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DETERMINATION OF ACETAZOLEAMIDE IN AQUEOUS HUMOR OF THE RABBIT EYE — HARRY GREEN, Ph.D., ET AL.

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APRIL 1955

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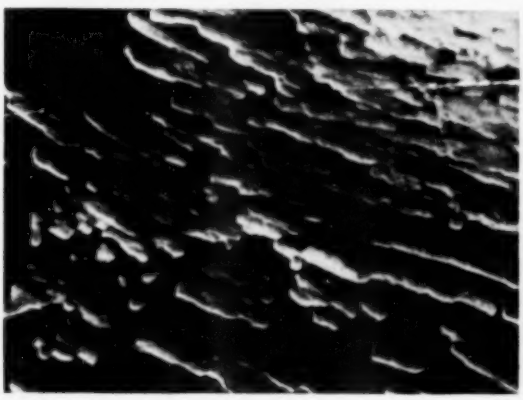

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+ or — .50	2.0 mm.	4.0 mm.
+ or — .75	1.3 mm.	2.6 mm.
+ or — 1.00	1.0 mm.	2.0 mm.
+ or — 1.25	.8 mm.	1.6 mm.
+ or — 1.50	.7 mm.	1.3 mm.
+ or — 1.75 to + or — 3.00	.5 mm.	1.0 mm.
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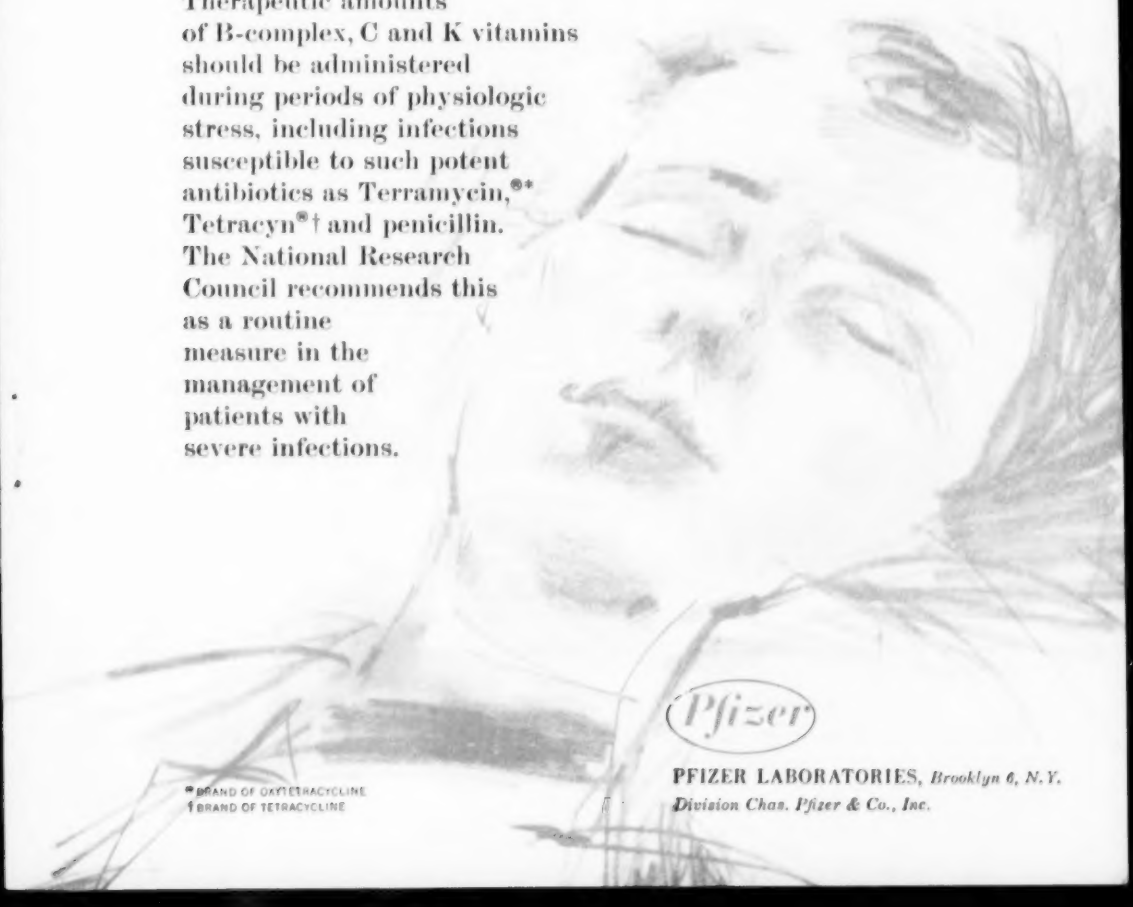

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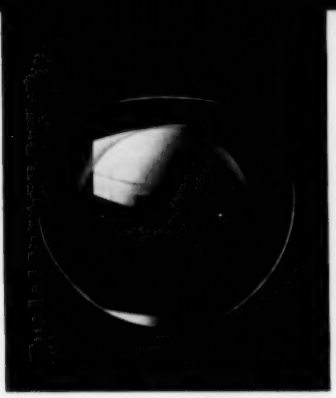

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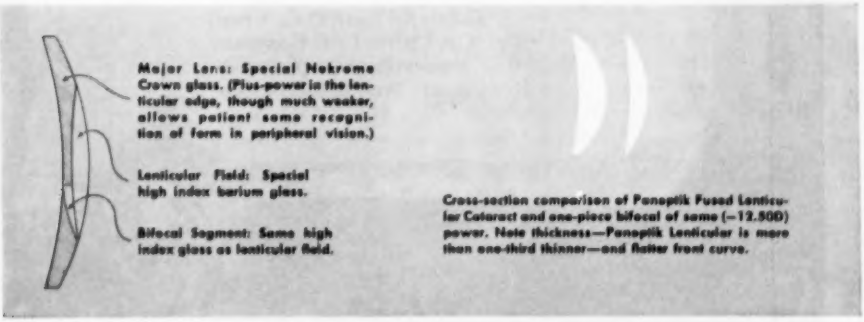
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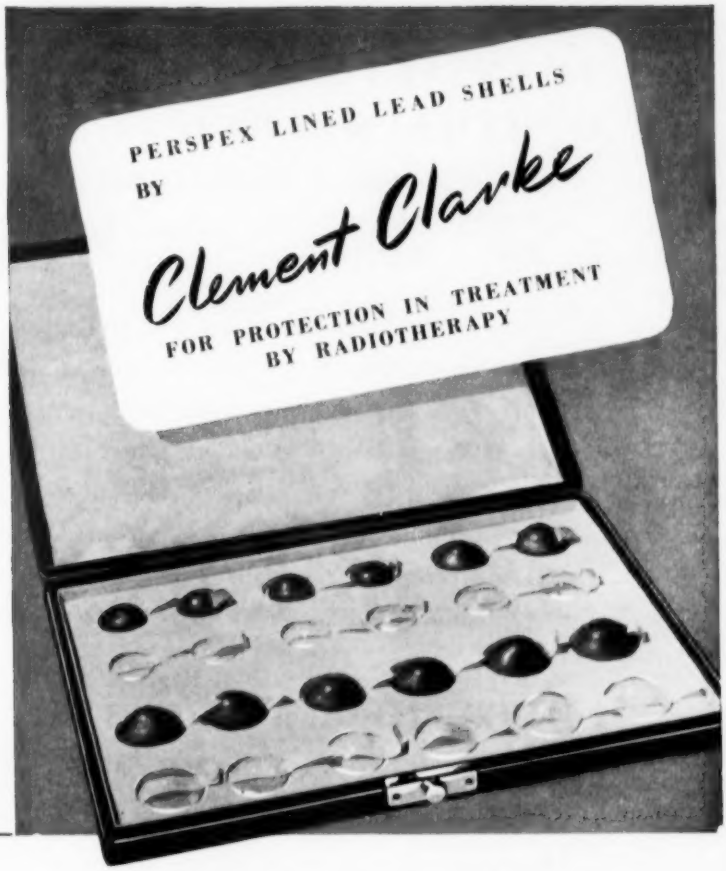

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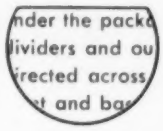

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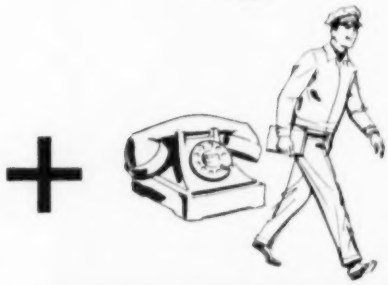

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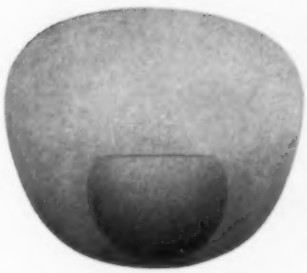

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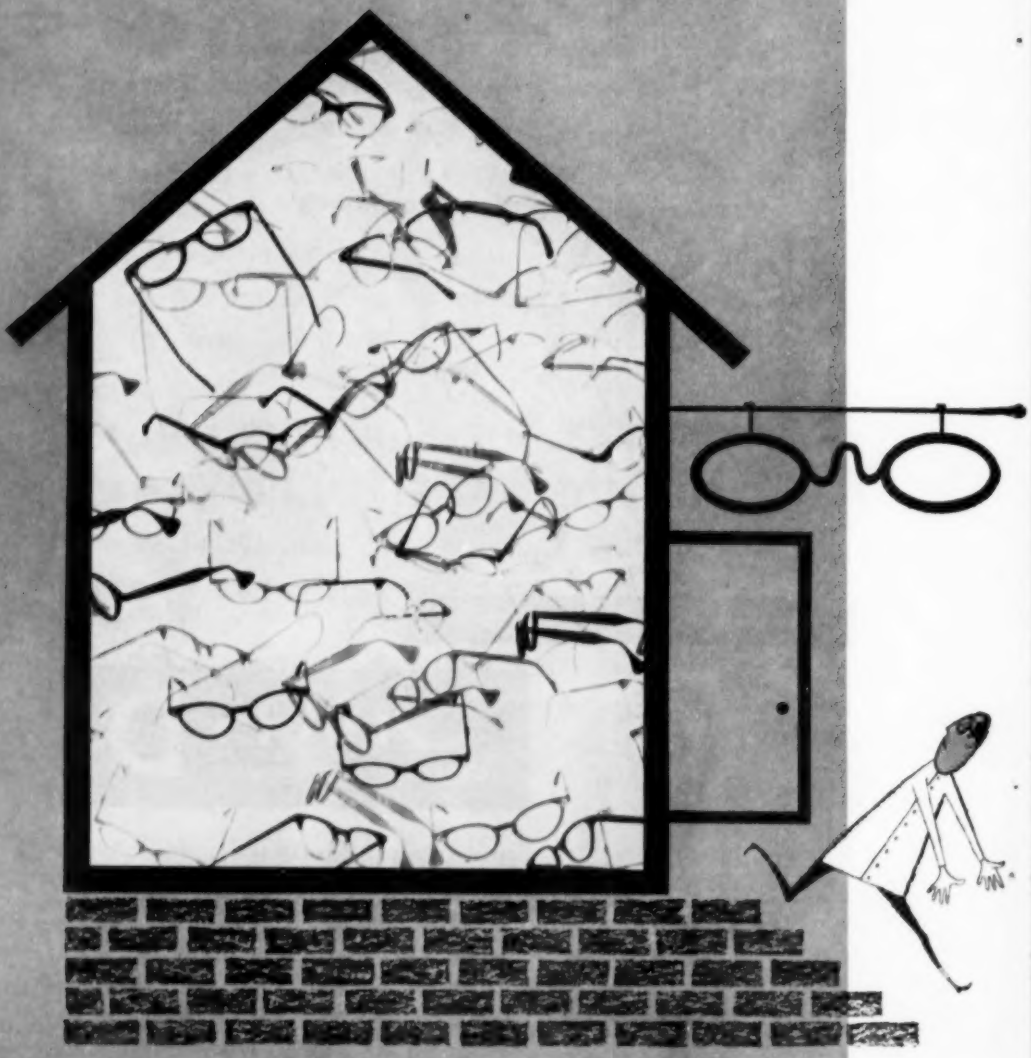

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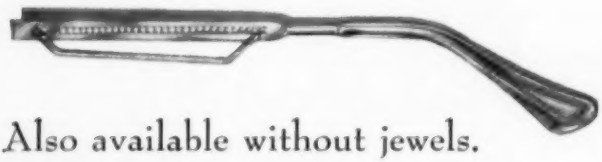

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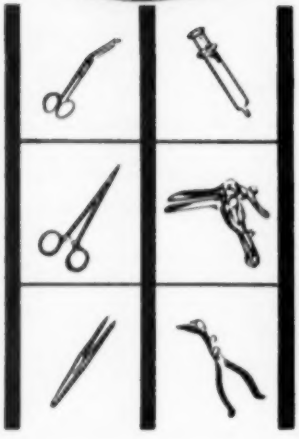

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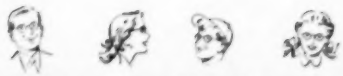

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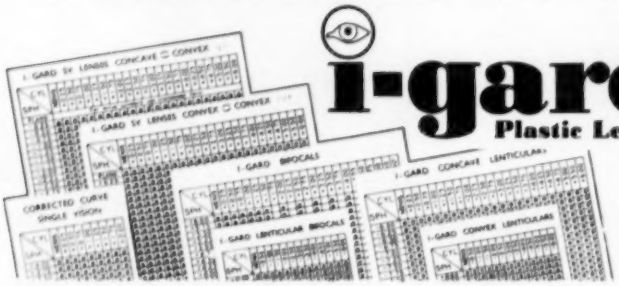

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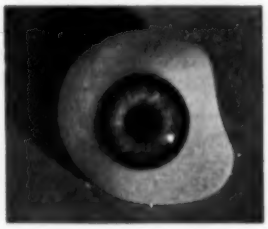

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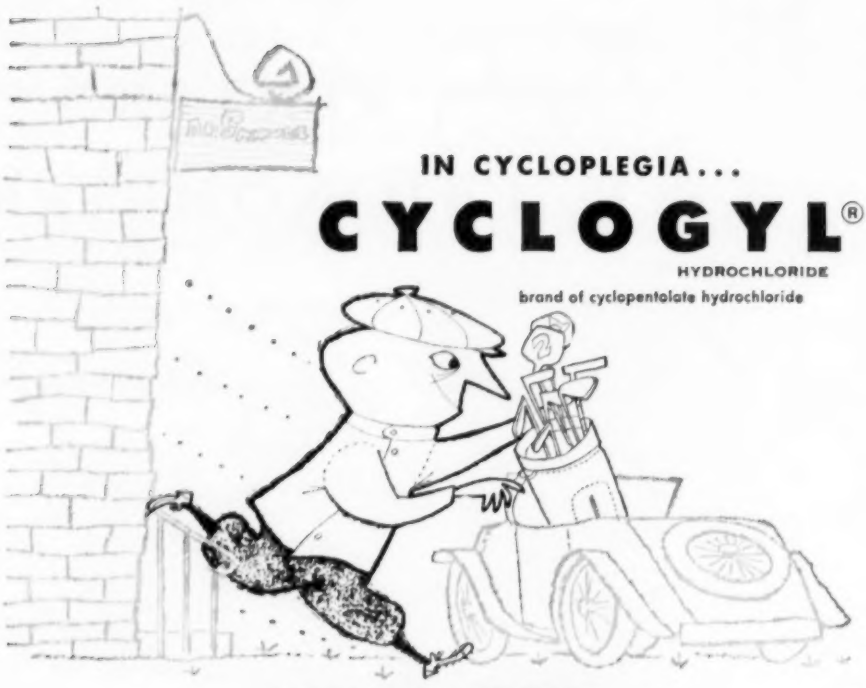

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*Successive drops at five minute intervals.

†Recovery time reduced to six hours after one percent pilocarpine.

1. *Gordon, D. M., and Ehrenberg, M. H.: Am. J. Ophth. 38:831 (Dec.) 1954.*

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Getter, B. C.: A.M.A. Arch. Ophth. 51:467 (Apr.) 1954.

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Carbonic Anhydrase and the Maintenance of Intraocular Tension

HARRY GREEN, Ph.D.
CAROL A. BOCHER, M.S.
ARTHUR F. CALNAN, M.D.
and
IRVING H. LEOPOLD, M.D., Philadelphia

With the Technical Assistance of Allen P. Rosenberg, B.S.

It had previously been reported [1] that the intravenous administration of acetazoleamide (Diamox; 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) inhibited * the carbonic anhydrase activity of the ciliary body-iris tissue within 30 minutes. This observation, together with a knowledge of the fundamental role of the enzyme in other tissues and cells, suggested the possibility that carbonic anhydrase may be the controlling mediator in the secretion of bicarbonate ions into the aqueous humor. In such a system the inhibition of the carbonic anhydrase activity in the anterior uvea should be reflected in a lowering of the bicarbonate ion concentration in the aqueous humor. Furthermore, according to Kinsey's postulate,[2] namely, that the bicarbonate ion plays a pivotal role in the formation of aqueous humor, there should also occur a drop in the intraocular pressure of the rabbit eye.

In order to test the possibility that the carbonic anhydrase activity of the ciliary body controlled the secretion of bicarbonate ions into the aqueous humor, and, therefore, regulated the intraocular pressure, a study of the relationship between the enzyme activity and these two phenomena was made.

The results to be reported show that the intravenous and subconjunctival injection of acetazoleamide completely inhibited the carbonic anhydrase activity of the ciliary body and iris tissue. At the same time the intraocular tension of a normal rabbit eye was unaffected. Similar results were obtained with the use of *p*-carboxybenzenesulfonamide (Dirnate), another carbonic anhydrase inhibitor.[3]

In the next paper [4] are reported the results of the study of the relationship between the enzyme activity and the concentration of bicarbonate ions in the aqueous humor.

EXPERIMENTAL METHODS

The sodium salt of acetazoleamide † was injected, 30 to 100 mg. per kilogram of body weight, into the marginal ear vein of adult pigmented rabbits, 5 to 6½ lb. (2.5 to 2.9 kg.) in weight. Unless otherwise indicated, the rabbits were supplied from the Bark-Bridge Farms, Wenonah, N. J. Subconjunctival injections into one eye were made with 0.2 ml. of a solution containing 5 to 20 mg. of the drug and previously neutralized to pH 8.8 to 9.0 in order to minimize secondary local tissue effects resulting from excessive alkalinity. With *p*-carboxybenzenesulfonamide,‡ 100 mg. in 0.5 ml. was injected intravenously, and 10 mg. in 0.2 ml. was injected subconjunctivally. Higher local concentrations produced marked chemosis and edema.

Intraocular Tension.—The test animal was either confined in a box lined with Foam Rubber with only its head protruding or restrained by holding its four paws with one hand while the other hand of the assistant firmly controlled the position of the head.§ The lids were held open with gentle pressure against the orbital rim. Measurements

From Wills Eye Hospital, Department of Research.

A preliminary report has appeared (Green, H., in discussion on Becker.[10]).

U. S. Public Health Service Research Fellow of the National Institute of Neurologic Diseases and Blindness under a Graduate Medical Training Grant (Dr. Calnan).

* The inhibition was complete, instead of 93%, as previously reported.

† Supplied by Dr. James D. Gallagher, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

‡ Dirnate, supplied by Dr. Gilbert Bayne, Sharp & Dohme Laboratories, West Point, Pa.

§ Technique recommended by Dr. E. J. Ballintine.

TABLE 1.—*Intraocular Tension of Normal, Untreated Rabbits**

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
6	6	6	5	7	5	6	5	5	4	+0.2	−0.6
5	6	7	6	7	8	8	7	7	7	+2.2	+1.2
7	5	..	..	3	3	5	3	4	4	−0.8	−0.2
7	7	..	..	4	3	3	4	4	3	−1.1	−1.2
5	5	..	..	3	7	3	6	2	8	−1.1	+1.0
6	7	..	..	4	4	5	3	3	2	−0.7	−1.9
7	4	..	..	3	6	3	3	5	4	−0.5	−0.4
4	3	..	..	3	2	3	5	4	3	+0.1	+0.4
6	5	6	7	4	6	6	5	.	..	−0.3	−2.8
7	6	5	6	5	4	3	3	.	.	−2.9	−1.1
7	6	5	4	5	5	3	4	.	..	−2.9	−1.5
7	5	3	5	7	6	5	3	.	.	−0.3	−3.0
7	8	4	4	5	3	5	4	..	.	−1.0	−4.2
7	9	8	5	5	6	6	3	.	..	−1.5	−0.5
5	6	4	2	5	3	5	6	..	..	+0.3	−4.2
								Average		−0.69	−1.27
								S. D.		0.90	1.29
								Total average		−0.98	
								Total S. D.		1.14	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye.

TABLE 2.—*Intraocular Tension After Intravenous Injection of Acetazolcamide, 30 Mg/Kg. Body Weight**

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
11	8	9	9	7	6	8	7	6	8	−2.5	−1.5
6	7	6	8	6	8	6	7	6	6	0.0	−0.1
8	6	8	7	6	6	5	5	6	5	−2.8	−1.1
6	6	6	6	7	7	4	3	6	7	−1.5	−2.3
6	7	6	8	7	8	8	6	4	5	+1.3	−1.0
8	6	8	6	8	7	7	6	8	7	−0.8	+0.2
6	7	7	7	6	7	7	7	6	6	+0.5	0.0
8	8	5	7	7	5	9	8	7	6	+1.7	−0.1
7	5	7	6	7	7	6	6	8	8	−0.8	+0.9
8	7	6	8	7	6	8	5	6	6	+0.5	−2.1
4	4	6	5	5	6	6	6	5	7	+1.2	+1.7
4	6	9	9	9	10	7	10	5	6	+1.7	+3.0
4	6	7	8	10	11	7	9	5	7	+2.5	+2.7
5	6	7	9	6	7	5	6	6	7	−0.5	−0.8
6	5	8	8	9	10	9	8	5	6	+2.3	+2.3
7	6	8	6	6	5	8	9	6	9	+0.3	+2.4
8	4	7	5	7	7	9	9	7	10	+1.0	+4.3
7	8	6	8	6	5	9	5	7	8	−1.5	−3.0
7	5	10	7	9	9	6	7	8	6	+1.8	+1.7
5	6	7	7	7	6	5	7	8	5	−0.3	+0.5
								Average		+0.22	+0.39
								S. D.		0.80	1.14
								Total average		+0.31	
								Total S. D.		1.00	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye.

were made every 30 minutes for a period of three hours with a Schiøtz tonometer, using a 5.5 gm. weight. All readings were made to the nearest scale unit. The eyes were anesthetized with 1 drop of 0.5% tetracaine (Pontocaine) hydrochloride. In a few cases, after subconjunctival injection, the intraocular pressure was measured by means of a phlebomanometer ‖ over a period of four hours.

‖ Measurements made by Dr. Enrique Wudka. The animals in the latter experiments were anesthetized with intravenous pentobarbital (Nembutal).

TABLE 3.—*Intraocular Tension After Intravenous Injection of Acetazoleamide, 100 Mg/Kg. Body Weight* *

Scale Readings								Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
5	6	8	7	7	8	6	8	+0.2	+1.7
6	4	5	6	5	6	7	6	0.0	+1.3
5	4	7	3	5	6	3	3	−2.3	−0.2
5	5	6	6	4	5	4	5	−1.3	−0.3
4	5	5	7	5	4	4	2	−0.2	−3.3
4	5	5	7	3	5	4	4	−0.5	−1.5
5	6	5	6	5	6	6	5	+0.8	−0.8
						Average		−0.47	−0.44
						S. D.		0.75	0.98
						Total average		−0.46	
						Total S. D.		0.91	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye.

TABLE 4.—*Intraocular Tension After Subconjunctival Injection of Acetazoleamide, 10 Mg. in 0.2 Ml.**

Scale Readings								Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
5	3	5	4	5	4	5	5	0.0	+1.5
3	2	3	4	4	4	5	3	+1.8	+0.5
3	2	5	4	4	3	4	4	+0.4	+1.2
3	7	6	8	3	4	5	4	+0.6	−3.3
3	3	4	3	4	3	6	5	+2.2	+1.7
5	3	5	7	5	4	5	3	+1.3	−1.1
4	2	4	6	6	4	5	4	+1.2	+0.7
4	5	6	4	4	3	5	3	+0.1	−1.7
2	4	4	3	3	3	5	4	+2.0	+0.1
4	3	6	4	5	6	5	4	+0.5	+1.0
						Average		+1.01	+0.06
						S. D.		0.76	0.83

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left (injected) eye; O. D., right (control) eye.

TABLE 5.—*Intraocular Tension of Rabbits After Intravenous Injection of p-Carboxybenzenesulfonamide, 100 Mg.**

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
5	6	5	5	4	5	4	5	5	4	−1.0	−0.7
5	4	4	4	5	3	4	6	4	4	−0.5	+1.5
5	6	6	5	6	7	6	8	9	4	+0.7	+2.1
4	5	5	6	6	5	6	6	4	4	+1.6	+0.5
6	7	7	8	8	7	8	6	10	11	+1.7	−1.1
5	4	5	6	5	4	7	8	8	6	+1.7	+2.7
6	6	9	7	9	8	8	8	8	9	+1.1	+1.6
8	7	8	10	10	9	9	9	10	8	+1.2	+1.0
7	9	9	7	7	7	7	8	8	6	−0.7	−0.5
6	5	7	6	6	5	6	5	6	6	−0.3	−0.3
								Average		+0.53	+0.78
								S. D.		0.47	0.73
								Total average		+0.66	
								Total S. D.		0.65	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye.

Carbonic Anhydrase Activity.—Treatment of the animal, the procedure for removing the ciliary body-iris tissue, and the method of assay of enzyme activity were essentially the same as previously described.[1]

RESULTS

Intraocular Tension.—The results, given in Tables 1 to 6, show the observed scale readings at the indicated time period. In order to assess the effect of the experimental treatment upon the intraocular tension over the experimental test period, rather than at each specified time, the observed scale readings were plotted and the straight line that best fits each set of points was graphically determined by the ingenious and simple method of Dr. Samuel Askovitz.¶ Briefly, the method starts with the plotting of an odd number of experimental observations obtained at equal intervals of time. Starting with the points on either side of the middle one, a line is drawn two-thirds the distance to the next point (away from the center); this line is then continued in the direction of the next point for the same horizontal distance. The terminal points of the two lines are connected by means of a straight line, the slope of which represents the mean rate of change of the data. It has been shown that this slope is identical to that obtained by fitting the data with the best possible straight line according to the criterion of least squares. A positive value indicates an increase in scale reading, and a negative value, a decrease. The individual slopes for each eye of each animal are given in the last two columns in Tables 1 to 6 and are stated in terms of change in number of tonometer scale units per 100 minutes. Besides the average and standard deviation of the values for each eye, there are also presented the average and standard deviation of all the eyes in a given experiment (total average; total σ).

In view of the fact that the error associated with reading the Schiøtz tonometer is generally considered to be at least one (1) scale unit, the evaluation of the physiologic importance of the experimental observations is considered relative to this error of measurement. In the usual methods of statistical comparison of data through mean values and standard deviations, it is assumed that the experimental measurements have been read with precision. Any inherent error in the recording of the data must therefore be considered together with the statistical evaluations.

It is clear from the data in Tables 1 to 6 that intravenous and subconjunctival administration of acetazoleamide and of *p*-carboxybenzenesulfonamide did not appreciably affect the intraocular tension of a normal rabbit. Similar results were obtained with the use of a phlebomanometer.

TABLE 6.—*Intraocular Tension of Rabbits After Subconjunctival Injection of 10 Mg. (0.2 Ml.) of p-Carboxybenzenesulfonamide* *

Scale Readings								Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
5	6	3	2	4	5	4	6	−0.3	+1.1
6	5	3	3	7	6	6	5	+1.1	+0.8
7	5	2	2	7	4	5	4	−0.1	0.0
5	6	5	3	6	3	4	6	+0.1	+0.5
5	5	3	2	6	5	5	4	+0.9	+0.2
						Average		+0.34	+0.52
						S. D.		0.41	0.39
						Total average		+0.43	
						Total S. D.		0.41	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye (both eyes injected).

¶ Askovitz.[5] Dr. Askovitz assisted in the statistical evaluation of the data.

Carbonic Anhydrase Activity.—The carbonic anhydrase activity of the ciliary body-iris tissue was completely inhibited 30 and 60 minutes after the intravenous administration of 30 mg. of acetazoleamide per kilogram of body weight and as early as 15 minutes after the subconjunctival administration of 10 mg. of the same drug. Similar results were obtained with the intravenous and subconjunctival administration of *p*-carboxybenzenesulfonamide. Since acetazoleamide is a potent carbonic anhydrase inhibitor—1.4×10^{-6} M solution completely inhibited the enzyme in an extract of one ciliary body-iris tissue #—it was of fundamental importance to establish whether the experimentally measured in vitro inhibition actually reflected the in vivo state of affairs or was due to the contamination of the extract by adsorption of small amounts of the drug in surface contact with the tissue. *

That a true in vivo inhibition of the enzyme activity occurred was shown by the following experiment: The ciliary body-iris tissue of an untreated normal rabbit was removed and immersed in two successive saline solutions that were used for the wash-by-immersion of a tissue whose carbonic anhydrase activity was completely inhibited as a result of the intravenous or subconjunctival injection of acetazoleamide. After immersion in fresh saline solution, the extract of the tissue was prepared and found to possess the original level of carbonic anhydrase activity previously found for normal tissue.

The possibility that the residual blood in the tissue contained sufficient acetazoleamide to inhibit the resulting extract is inconsistent with the facts. At least 1γ of the drug is necessary to inhibit the enzyme activity completely in 1 ml. of the extract containing one tissue. Since the residual blood in the tissue amounts to 0.0008 ml.,[1] a concentration of 1.25 mg/ml. of blood in the animal would be necessary. Assuming 250 to 300 ml. to represent the average blood volume of a 5 to 6 lb. (2.3 to 2.7 kg.) rabbit, 312.5 to 375.0 mg. of the drug must be present in the circulatory system of the animal. The absurdity of this is obvious, since as little as 10 mg. injected subconjunctivally, or less than 100 mg. per animal (30 mg/kg. body weight given intravenously) produced complete inhibition of the enzyme activity.

Distribution of Carbonic Anhydrase Activity in Anterior Uvea.—In view of the physiologic importance of the ciliary body as the seat of the secretory mechanism, it was desirable to establish whether acetazoleamide actually inhibited the carbonic anhydrase activity of the ciliary body. For this purpose the ciliary body was separated from the iris according to the technique of Friedenwald, Herrmann, and Moses,[8] after the eye was enucleated as previously described.[1]

TABLE 7.—*Distribution of Carbonic Anhydrase Activity in the Anterior Uvea* *

Experiment	Iris μL CO_2	Ciliary Body μL CO_2	Total μL CO_2
C-55	11.75	21.33	33.08
C-57	10.90	13.19	24.09
C-66	12.14	16.27	28.41
Average	11.60	16.93	28.53†

* Values expressed as microliters CO_2 liberated from a $NaHCO_3$ solution in 30 seconds at 0.5-2 C. Each experiment is an average of three sample aliquots.

† The activity of the ciliary body-iris tissue as a whole was 25 to 30 μL CO_2/30 sec. (Green and associates [1]).

The separated tissues were homogenized in phosphate buffer, pH 7.0, so that 1 ml. of resulting extract contained the equivalent of one tissue. The assay of the enzyme activity is given in Table 7 and shows that the sum of the individual activities equals that of the whole and that 60% of the total activity is present in the ciliary body. The enzyme in each tissue was completely inhibited by the intravenous injection of 30 mg. of acetazoleamide per kilogram of body weight in 30 minutes and after the subconjunctival injection of 10 mg. of the drug.

It is interesting to note that in those cases in which one eye of the animal was used as a control for the subconjunctival injection of the other eye, the carbonic anhy-

Lederle investigators,[6] working with the enzyme obtained from beef erythrocytes, reported a value of 7.8×10^{-7} M as the minimum concentration of acetazoleamide for complete inhibition.

* Measurable amounts of the drug were found in the aqueous humor.[7]

drase activity of the ciliary body-iris tissue of the uninjected eye was inhibited approximately 50% in 30 minutes.

COMMENT

On the basis of the observations of the beneficial effects of acetazoleamide in the lowering of intraocular pressure of glaucomatous eyes, it was reasonable to assume that the drug exerted its therapeutic properties by inhibiting the carbonic anhydrase activity of the ciliary body, thereby decreasing the rate of elaboration and active transfer of bicarbonate ions into the aqueous humor. This investigation is the first to present direct experimental evidence of the effect of local and systemic use of acetazoleamide upon the carbonic anhydrase activity of the anterior uvea of the rabbit eye.

The results of these studies clearly indicate that the local and systemic administration of the carbonic anhydrase inhibitors acetazoleamide and *p*-carboxybenzenesulfonamide completely inhibited the carbonic anhydrase activity of the ciliary body and iris of the rabbit eye. At the same time no significant lowering of the intraocular tension was observed. On the basis of these observations, it is reasonable to conclude that the maintenance of the intraocular tension of a normal rabbit eye is not dependent upon the carbonic anhydrase activity of the anterior uvea.

While this investigation was in progress and shortly after its completion, it became apparent from the experience of other investigators that general agreement was lacking concerning the effect of intravenously administered acetazoleamide on the intraocular pressure of a normal rabbit eye. Thus, Grant and Trotter,[9] in preliminary experiments, observed a definite lowering of the intraocular pressure when 10 to 100 mg. of the drug was administered per kilogram of body weight. Becker[10] obtained a definite drop in about 90% of the rabbits tested,† while Ballintine ‡ observed a drop in only 50% of the rabbits tested. Harris,§ on the other hand, has not observed any definite effect of the drug on the intraocular pressure of the normal rabbit eye. In these cases, however, the carbonic anhydrase activity of the anterior uvea was not reported.

On the other hand, there is unanimous agreement among the investigators that subconjunctival injection of acetazoleamide produced no significant lowering of the intraocular pressure of a normal rabbit eye.

SUMMARY

Of the total carbonic anhydrase activity of the anterior uvea of the rabbit eye, the ciliary body possesses 60% and the iris 40%.

Intravenous and subconjunctival administration of acetazoleamide (Diamox; 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) completely inhibited the carbonic anhydrase activity in the ciliary body and iris of the rabbit eye.

The inhibition was shown to be a true in vivo reaction, and not caused by contamination by the drug adsorbed on the tissue or present in the residual blood in the tissue.

No significant lowering of the intraocular tension of the rabbit eye was observed after

† Experiments similar to the ones reported herein were performed with rabbits obtained from the Haskins Rabbitry in St. Louis, the same rabbitry which supplied the "responsive" animals used by Becker.[10] In our hands these animals showed no appreciable lowering of the intraocular tension after systemic administration of acetazoleamide (Tables 8 and 9). The same animals after bilateral nephrectomy, when given the drug intravenously 18 to 20 hours later, showed a significant fall in the intraocular tension (Table 10), in agreement with Becker. Similar experiments with our usual rabbits showed no lowering in intraocular tension (Tables 11 and 12). In all cases the carbonic anhydrase activity of the anterior uvea was inhibited. It is significant, moreover, to note that we have experimentally found that after intravenous administration the concentration of acetazoleamide in the blood plasma of the nephrectomized rabbit was considerably in excess of that required to inhibit completely the carbonic anhydrase activity in the erythrocytes. Under these conditions interference with the respiratory mechanism of gas exchange may very well account for the local ocular effects observed by Becker with nephrectomized rabbits.

‡ Ballintine, E. J.: Personal communication to the authors.

§ Harris, J. L., in discussion on Becker.[10]

TABLE 8.—*Intraocular Tension of Rabbits from Haskins Rabbitry, St. Louis, After Intravenous Injection of Acetazolcamide, 30 Mg/Kg. Body Weight* *

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
5	5	5	4	4	4	6	4	5	4	+0.6	—0.6
3	3	4	4	5	6	6	5	7	8	+2.5	+1.9
5	4	6	4	6	4	7	6	7	5	+1.5	+1.7
3	4	6	3	3	5	6	5	6	5	+1.5	+1.4
4	3	5	4	5	6	11	6	11	5	+5.7	+2.7
5	4	5	6	3	4	3	4	5	4	—2.0	—0.7
4	3	4	5	5	6	6	5	5	5	+1.8	+1.6
3	3	5	4	5	4	4	5	5	6	+0.5	+1.5
5	4	6	5	6	5	6	6	4	8	+0.7	+1.5
4	5	6	5	3	3	5	4	6	4	0.0	—1.2
								Average		+1.28	+0.98
								S. D.		1.53	0.56
								Total average		+1.08	
								Total S. D.		1.16	
Control Rabbits—Uninjected											
5	4	5	3	6	4	5	5	5	5	+0.2	+1.1
4	5	3	3	3	2	6	5	5	5	+1.9	+0.2
								Average		+1.05	+0.65
								Total average		+0.85	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye.

TABLE 9.—*Intraocular Tension of Rabbits from Haskins Rabbitry, St. Louis, After Intravenous Injection of Acetazolcamide, 30 Mg/Kg. Body Weight: Same Animals as Those in Table 8, Treated Forty-Eight Hours Later* *

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
3	3	5	5	6	5	5	6	5	6	+1.3	+2.0
5	4	7	7	5	6	7	6	7	5	+1.4	+1.0
5	4	5	6	4	5	4	4	5	6	—1.0	—0.4
4	6	5	6	7	4	7	5	6	5	+2.7	—1.1
3	6	7	6	6	6	5	4	5	3	+0.8	—0.8
6	5	5	6	9	7	8	8	7	8	+2.6	+2.4
5	3	5	4	4	4	5	6	5	4	—0.2	+2.3
6	6	6	7	5	6	5	3	4	5	—1.0	—2.8
5	4	5	6	6	5	6	5	5	5	+1.0	+0.3
4	3	7	7	4	3	5	4	5	4	—0.2	—0.4
								Average		+0.74	+0.25
								S. D.		0.80	0.99
								Total average		+0.50	
								Total S. D.		0.85	
Control Rabbits—Uninjected											
5	6	4	4	3	5	4	5	5	5	—0.8	—0.4
3	4	3	4	4	4	4	6	5	4	+1.0	+1.6
								Total average		+0.37	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left eye; O. D., right eye.

TABLE 10.—*Intraocular Tension of Rabbits from Haskins Rabbitry, St. Louis, Having Received Intravenous Acetazoleamide, 30 Mg/Kg. Body Weight Eighteen to Twenty Hours After Bilateral Nephrectomy* *

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
4	5	7	7	7	8	8	10	7	8	+2.8	+4.0
5	4	9	8	8	9	8	9	8	9	+1.6	+5.4
5	5	10	9	11	10	16	12	13	14	+8.5	+4.8
4	4	5	6	7	8	8	8	7	5	+3.0	+3.6
3	5	10	11	10	11	13	12	9	11	+7.1	+3.4
5	6	8	7	8	8	8	9	5	8	+2.0	+2.5
								Average		+4.2	+4.0
								S. D.		2.6	0.95
								Total average		+4.1	
								Total S. D.		1.98	
Control Rabbits—Uninjected											
6	7	5	6	6	5	5	6	5	5	−0.5	−0.8
4	5	4	3	5	3	5	4	4	3	+1.0	−0.5
								Average		+0.25	−0.65
								Total average		−0.2	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight, O. S., left eye; O. D., right eye.

TABLE 11.—*Intraocular Tension of Rabbits Having Received Intravenous Acetazoleamide, 30 Mg/Kg. Body Weight, Eighteen to Twenty Hours After Bilateral Nephrectomy* *

Scale Readings										Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		60 Min.		120 Min.		180 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
9	8	7	7	7	8	9	8	9	10	+0.4	+0.3
7	7	8	7	8	7	8	8	9	9	+0.7	+0.8
8	10	8	7	7	8	9	7	8	9	+0.7	−1.8
8	8	9	10	9	10	9	9	10	9	−0.2	+0.5
5	5	6	7	7	6	5	7	7	8	0.9	+1.1
								Average		+0.32	+0.18
								S. D.		0.28	0.53
								Total average		+0.25	
								Total S. D.		0.49	
Control Rabbits—Uninjected											
7	9	6	8	7	8	7	6	7	7	+0.3	−2.3
7	7	7	7	8	8	6	8	7	7	−0.7	+1.0
								Average		−0.2	−0.65
								Total average		−0.43	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight, O. S., left eye; O. D., right eye.

TABLE 12.—*Intraocular Tension of Rabbits Having Received 10 Mg. (0.2 Ml.) Acetazoleamide Subconjunctivally Eighteen to Twenty Hours After Bilateral Nephrectomy* *

Scale Readings								Average Slope Scale Units/100 Min.	
0 Min.		30 Min.		90 Min.		150 Min.			
O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.
7	6	4	5	6	5	6	7	0.0	+1.0
6	5	6	5	6	5	5	7	−0.8	+1.7
6	7	5	5	5	6	5	6	−0.7	−0.3
4	7	5	7	5	7	7	6	+2.3	−0.8
						Average		+0.20	+0.40
						S. D.		0.84	0.50
						Total average		+0.30	
						Total S. D.		0.69	

* Values are scale readings with Schiøtz tonometer, 5.5 gm. weight. O. S., left (injected) eye; O. D., right (control) eye.

local and systemic administration of acetazoleamide.

Similar results were obtained with the use of *p*-carboxybenzenesulfonamide (Dirnate).

The results indicate that the maintenance of the intraocular pressure of the normal rabbit eye is not dependent upon the carbonic anhydrase activity of the anterior uvea.

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Carbonic Anhydrase and the Elaboration of Bicarbonate Ion in the Rabbit Eye

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In the preceding paper [2] evidence was presented that the maintenance of intraocular tension of a normal rabbit eye was not dependent upon the carbonic anhydrase activity of the anterior uvea. The present study was undertaken to determine whether the inhibition of carbonic anhydrase activity of the anterior uvea by the intravenous and subconjunctival administration of acetazoleamide (Diamox; 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) affected the concentration of bicarbonate ions in the aqueous humor. Our results show that the elaboration and active transfer of bicarbonate ions into the aqueous humor are not mediated by the carbonic anhydrase of the anterior uvea of the normal rabbit eye.

EXPERIMENTAL STUDY

The recent observations of Kinsey [3] that the concentration of bicarbonate ions in the posterior chamber of the rabbit eye is not only greater than that in the plasma but also greater than that in the anterior chamber were offered in support of Friedenwald's postulate [4] that bicarbonate ion is secreted by the ciliary body. Kinsey also suggested that the difference in bicarbonate ion concentration in the two chambers is due to a net flow from the anterior chamber to the plasma and that the mechanism of this transfer is by a process no more complex than simple diffusion. Because of these considerations it was felt that any interference with the active transfer of bicarbonate ion into the posterior chamber would be reflected in a lowering of the concentration of the ion in the anterior chamber. The net result of the inhibition of the secretion of bicarbonate ions into the posterior chamber would be physical equilibration of the bicarbonate ion between the plasma and the anterior aqueous humor * by virtue of the passive and reversible nature of diffusional transfer. For these reasons it was decided to determine the effect of acetazoleamide upon the bicarbonate ion concentration in the more readily accessible and abundant anterior chamber aqueous humor. In all cases a sample of blood plasma prepared from blood withdrawn at the same time as the anterior chamber was tapped was analyzed for bicarbonate concentration.

Methods and Procedures

The bicarbonate ion concentration was determined in a Warburg apparatus at 37 C in an atmosphere of 95% N_2 and 5% CO_2 by measuring the CO_2 liberated by H_2SO_4. In all cases 0.15 to 0.20 ml. of anterior aqueous humor or blood plasma was placed in the main compartment of a Warburg vessel, equilibrated, and gassed for 10 minutes at 37 C. With the manometer adjusted for the zero minute reading, 0.2 ml. of 0.1N H_2SO_4 was tipped in from the side-arm. The reaction was complete within 5 to 10 minutes.

One adult pigmented rabbit, weighing 5½ to 6½ lb., was used in each experiment. Immediately

From the Wills Eye Hospital, Department of Research.

A preliminary report has appeared (Green, H., in discussion on Becker [1]).

* Nomenclature suggested by Kinsey.[3]

prior to the administration of the drug,† aqueous humor was removed from one eye and a sample of cardiac blood obtained to serve as controls. Intravenous and subconjunctival injections were done as previously described. At the specified time period the eye was anesthetized with tetracaine hydrochloride, flushed thoroughly with isotonic saline solution, and proptosed. The anterior aqueous humor was removed by an oblique corneal-limbal puncture through a 26-gauge needle either into a tuberculin syringe or into a graduated pipette fitted onto the needle. In the latter case the aqueous humor was measured directly into the Warburg flask; in the former case it was first emptied into a conical tube, from which the desired amount was pipetted into the Warburg flask. Immediately after paracentesis of the anterior chamber a blood sample was obtained by cardiac puncture with a syringe wet with heparin. The plasma was obtained after centrifugation at 0 C. at 2800 rpm for 10 minutes in an International Refrigerated Centrifuge, PR-1.

by saturating the system with a gas atmosphere of 5% CO_2 at 37 C and atmospheric pressure, the concentration in the original body fluid may be directly measured with an accuracy of about 95%.

Normal Variation Between Eyes.—Before investigating the effect of experimental conditions upon the normal composition of an animal eye, using the companion eye as a control, it is necessary to determine, first, the difference in concentrations of the particular substance that may normally exist between the two eyes, and, second, whether this difference changes during the experimental period. The results in Table 1 show

TABLE 1.—*Variation of Bicarbonate Ion Concentration in Aqueous Humor of Normal Rabbit Eyes**

	0 Min. O. S.	0 Min. O. D.	Diff.	0 Min. O. S.	30 Min. O. D.	Diff.	0 Min. O. S.	60 Min. O. D.	Diff.
	27.7	31.1	+3.4	23.0	24.4	+1.4	33.5	33.0	−0.5
	25.6	23.1	−2.5	38.4	35.8	−2.6	34.2	32.0	−2.2
	33.2	32.6	−0.6	26.4	28.2	+1.8	32.0	31.9	−0.1
	28.8	30.0	+1.2	28.8	30.4	+1.6	24.4	23.3	−1.1
	29.9	30.9	+1.0	25.5	31.2	+5.7	27.0	27.3	+0.3
	29.6	26.4	−3.2	27.0	28.6	+1.6	26.7	31.0	+4.3
	26.6	25.8	−0.8	31.4	27.8	−3.6	34.8	32.5	−2.3
	26.8	27.0	+0.2	31.8	27.7	−4.1	29.6	28.7	−0.9
	30.4	29.8	−0.6	38.6	41.0	+2.5	31.0	30.2	−0.2
Av.	28.7	28.5	1.5	30.1	30.6	2.8	30.4	30.0	1.3
S. D.	2.6	3.1	1.1	5.2	4.7	1.4	3.1	2.8	1.3

Total average difference, 1.9 mM/L.
Difference greater than 2 mM/L., 26%.
Difference greater than 3 mM/L., 11%.

* Values are expressed in millimoles per liter.

RESULTS

Before assessing the physiologic significance of the experimental data, it is desirable to consider some fundamental characteristics of the system under investigation.

Accuracy of the Method.—The conventional Warburg technique of measuring gas exchange inherently possesses an accuracy of 95%, under conditions of high precision. The described method of determining the bicarbonate ion involves the assumption that, that in nine animals tested, the difference in bicarbonate ion concentration between the two eyes is, on the average, 1.5 mM/liter, and that this difference is not significantly affected by increasing the time of paracentesis of the second eye up to 60 minutes. In the 27 animals examined, therefore, the concentration of bicarbonate ion in the two eyes differs, on the average, by 1.9 mM/liter, during the course of 60 minutes; 26% of the animals gave a drop greater than 2 mM/liter, while 11% showed a fall in bicarbonate ion concentration greater than 3 mM/liter.

Intravenous Injection.—A. Aqueous Humor: Preliminary observations‡ indicated that a simultaneous and parallel lowering of the bicarbonate ion concentration in the blood

† Acetazoleamide was supplied by Dr. James D. Gallagher, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

‡ Green, H., in discussion on Becker.[1]

TABLE 2.—*Bicarbonate Ion Concentration After Intravenous Injection of Acetazoleamide, 30 Mg/Kg. Body Weight* *

	Plasma		Aqueous Humor		Ratio	
	0 Min.	30 Min.	0 Min.	30 Min.	0 Min.	30 Min.
	23.4	23.2	32.4	38.4	1.38	1.66
	17.8	20.8	34.1	23.8	1.91	1.14
	24.2	24.8	34.2	36.6	1.41	1.47
	30.8	24.6	25.7	28.7	0.87	1.20
	28.6	31.1	37.8	32.1	1.32	1.03
	32.4	27.2	33.5	31.6	1.03	1.16
	18.9	23.1	39.6	27.5	2.09	1.19
	18.6	25.2	32.5	27.8	1.75	1.10
	21.5	23.8	30.1	30.1	1.40	1.27
	20.1	16.4	29.1	25.3	1.45	1.54
	26.6	20.4	30.1	26.0	1.13	1.27
	19.6	20.0	30.1	27.8	1.53	1.39
	20.7	21.3	27.0	28.0	1.30	1.31
	19.8	23.9	30.8	32.0	1.55	1.34
	20.4	20.9	30.9	28.9	1.51	1.38
	20.4	23.8	29.6	31.9	1.45	1.34
	20.5	21.2	32.3	27.6	1.57	1.30
	19.5	20.3	32.0	27.2	1.64	1.34
	15.6	18.0	22.4	22.9	1.43	1.27
	20.3	19.9	21.1	26.5	1.04	1.33
	13.7	15.6	23.2	23.5	1.70	1.50
	17.3	18.6	28.2	23.5	1.63	1.26
	16.4	19.3	23.6	22.1	1.44	1.15
	23.0	22.6	29.7	24.6	1.29	1.11
Av.	21.3	21.9	30.2	28.1	1.46	1.29
S. D.	4.5	3.1	4.1	4.1	0.22	0.17
t	0.54		1.77		1.0	

* Time, 30 minutes; values expressed as millimoles per liter.

plasma and aqueous humor occurred 30 minutes after the injection of 30 mg. of acetazoleamide per kilogram of body weight of rabbit. The more extensive and statistically

TABLE 3.—*Bicarbonate Ion Concentration After Intravenous Injection of Acetazoleamide, 60 Mg/Kg. Body Weight* *

	Plasma		Aqueous Humor		Ratio	
	0 Min.	30 Min.	0 Min.	30 Min.	0 Min.	30 Min.
	25.4	22.4	31.3	29.8	1.23	1.33
	21.4	18.7	38.1	29.6	1.78	1.58
	18.0	22.8	32.5	29.2	1.80	1.28
	13.0	17.6	29.4	28.8	2.26	1.63
	15.1	22.4	30.6	28.5	2.02	1.27
	15.3	20.4	28.4	22.7	1.85	1.11
	18.0	24.1	30.7	26.1	1.70	1.08
	17.3	24.1	27.6	24.4	1.60	1.01
	17.9	29.8	25.1	22.7	1.40	0.76
	21.6	23.4	30.8	27.2	1.42	1.16
	13.8	19.5	25.9	24.2	1.88	1.24
	18.6	19.9	29.8	24.8	1.60	1.25
	14.7	19.1	27.7	27.2	1.88	1.42
Av.	17.7	21.8	30.5	26.6	1.73	1.24
S. D.	3.3	3.1	4.1	2.5	0.25	0.22
t	3.27		2.97		4.89	

* Time, 30 minutes; values expressed as millimoles per liter.

TABLE 4.—*Bicarbonate Ion Concentration After Intravenous Injection of Acetazoleamide, 100 Mg/Kg. Body Weight* *

	Plasma		Aqueous Humor		Ratio	
	0 Min.	5 Min.	0 Min.	5 Min.	0 Min.	5 Min.
	25.0	26.0	31.3	31.8	1.25	1.22
	22.8	26.2	35.6	30.4	1.56	1.16
	16.1	23.8	31.0	29.5	1.92	1.24
	19.2	26.5	36.5	34.5	1.90	1.30
	15.5	21.0	33.2	27.6	2.14	1.31
Av.	19.7	24.7	33.5	30.8	1.75	1.25
S. D.	3.7	2.0	2.1	2.3	0.33	0.10
t	2.677		1.992		3.330	

* Time, 5 minutes; values expressed as millimoles per liter.

evaluated results § are given in Tables 2 to 5. It can be seen that the injection of 30 mg. of the drug per kilogram of body weight did not significantly lower the bicarbonate ion concentration in the two fluids after 30 minutes (Table 2). The data in Table 3, however, indicate that the drop in bicarbonate ion concentration in the aqueous humor 30 minutes after the injection of 60 mg. of the drug per kilogram of body weight is statistically significant. Despite this mathematical fact, it is questionable whether such a slight drop (1.9 mM/liter greater than the difference that normally exists between the two eyes) is directly associated with the physiologic action of acetazoleamide, since the injection of a higher dose, 100 mg. per kilogram of body weight, did not appreciably af-

§ Dr. Askowitz assisted in the statistical evaluation of the data.

TABLE 5.—*Bicarbonate Ion Concentration After Intravenous Injection of Acetazoleamide, 100 Mg/Kg. Body Weight* *

	Plasma		Aqueous Humor		Ratio	
	0 Min.	60 Min.	0 Min.	60 Min.	0 Min.	60 Min.
	21.6	20.4	28.1	25.7	1.30	1.26
	20.5	20.8	27.2	25.2	1.33	1.69
	14.9	18.0	26.2	24.6	1.75	1.37
	25.9	21.7	35.2	26.9	1.36	1.24
	25.4	20.0	31.6	....	1.24	....
	23.4	25.2	33.5	30.6	1.40	1.20
	29.0	23.1	32.0	35.6	1.10	1.50
	28.4	30.4	35.2	35.6	1.20	1.20
Av.	23.6	22.5	31.1	30.6	1.34	1.35
S. D.	4.3	3.6	3.3	4.6	0.16	0.20
t	0.550		0.250		0.125	

* Time, 60 minutes; values expressed as millimoles per liter.

TABLE 6.—*Bicarbonate Ion Concentration After Subconjunctival Injection of Acetazoleamide, 5 Mg. in 0.2 Ml.**

	Plasma		Aqueous Humor		Ratio	
	0 Min.	60 Min.	0 Min.	60 Min.	0 Min.	60 Min.
	13.0	14.4	30.6	28.8	2.36	2.00
	13.5	16.5	35.0	31.3	2.59	2.00
	14.5	12.5	33.3	30.4	2.30	2.43
	20.2	13.5	26.8	30.0	1.33	2.22
	12.9	19.1	29.7	28.6	2.29	1.50
	14.6	16.8	30.6	28.3	2.10	1.68
Av.	14.8	15.5	31.0	29.6	2.16	1.97
S. D.	2.4	2.0	2.8	1.3	0.40	0.32
t	0.547		1.240		0.950	

* Time, 60 minutes; values expressed as millimoles per liter.

TABLE 7.— *Bicarbonate Ion Concentration After Subconjunctival Injection of Acetazoleamide, 10 Mg. in 0.2 Ml.**

	Plasma		Aqueous Humor		Ratio	
	0 Min.	30 Min.	0 Min.	30 Min.	0 Min.	30 Min.
	21.2	16.1	24.2	25.0	1.14	1.55
	20.3	15.5	27.1	26.7	1.33	1.72
	20.0	23.6	26.8	29.1	1.34	1.23
	30.0	22.8	32.9	35.4	1.10	1.55
	24.6	21.2	27.0	30.6	1.10	1.44
Av.	23.2	19.8	27.6	29.4	1.20	1.50
S. D.	3.9	3.6	2.9	3.2	0.10	0.10
t	1.434		0.932		5.00	

* Time, 30 minutes; values expressed as millimoles per liter.

fect the bicarbonate ion concentration in the aqueous humor (Tables 4 and 5).

B. Blood Plasma: It is clear from the results in Tables 2 to 4 that the systemic administration of 30 to 100 mg. of acetazoleamide per kilogram of body weight ‖ did not cause an appreciable lowering of the bicarbonate ion concentration in the blood plasma during the first 60 minutes. Indeed, as the results in Table 3 indicate, 30 minutes after the injection of 60 mg. of the drug per kilogram of body weight a significant increase in the bicarbonate ion concentration occurred (4.1 mM/liter). A similar increase (5.0 mM/liter) was noted five minutes after the injection of 100 mg. per kilogram of body weight (Table 4). The physiologic significance of the increase shown in Table 3 is minimized in consideration of the fact that 100 mg. per kilogram of body weight had no effect upon the bicarbonate ion concentration after 60 minutes (Table 5). The increase observed in Table 4 may be associated with an immediate, but only temporary, increase in pH of the blood plasma because of the relatively high concentration of alkalinity introduced (100 mg. of acetazoleamide per kilogram of body weight, at a pH of 10.0).

Subconjunctival Injection.—A. Aqueous Humor: The results are given in Tables 6 to 11 and show that the only statistically significant fall (3.8 mM/liter) in bicarbonate

TABLE 8.—*Bicarbonate Ion Concentration After Subconjunctival Injection of Acetazoleamide, 10 Mg. in 0.2 Ml.**

	Plasma		Aqueous Humor		Ratio	
	0 Min.	60 Min.	0 Min.	60 Min.	0 Min.	60 Min.
	18.9	19.7	29.8	32.4	1.58	1.64
	19.8	25.4	31.5	29.7	1.59	1.17
	22.7	25.6	36.2	34.4	1.60	1.34
	28.0	22.8	39.0	34.2	1.39	1.50
	20.8	20.8	33.6	34.2	1.61	1.65
	20.7	23.5	35.0	28.8	1.69	1.23
	25.6	26.0	32.4	27.7	1.29	1.07
	18.5	21.6	33.8	30.1	1.83	1.39
	18.8	23.4	35.4	24.4	1.88	1.04
	15.1	22.0	31.1	24.7	2.06	1.12
	12.5	22.8	30.4	25.4	2.43	1.11
	16.2	23.7	28.5	26.1	1.76	1.10
	22.8	20.2	30.1	28.1	1.32	1.39
	19.7	14.3	29.9	25.4	1.52	1.66
	23.8	15.8	34.4	25.1	1.45	1.59
	23.4	16.0	28.1	26.2	1.20	1.64
	30.9	18.6	31.9	29.9	1.03	1.60
Av.	21.0	21.3	32.4	28.6	1.60	1.37
S. D.	4.7	3.4	3.1	3.8	0.33	0.17
t	0.214		3.22		0.255	

* Time, 60 minutes; values expressed as millimoles per liter.

TABLE 9.—*Bicarbonate Ion Concentration After Subconjunctival Injection of Acetazoleamide, 10 Mg. in 0.2 Ml.**

	Plasma		Aqueous Humor		Ratio	
	0 Min.	180 Min.	0 Min.	180 Min.	0 Min.	180 Min.
	18.5	25.3	28.2	26.4	1.52	1.04
	15.7	26.3	33.2	31.0	2.11	1.18
	11.9	24.5	28.3	27.9	2.42	1.14
	21.6	21.3	30.7	25.8	1.42	1.21
	23.5	21.1	29.3	27.9	1.25	1.32
Av.	18.2	23.7	30.0	27.8	1.74	1.18
S. D.	4.1	2.1	2.4	1.8	0.45	0.10
t	2.625		1.654		2.860	

* Time, 180 minutes; values expressed in millimoles per liter.

‖ Doses up to 10 mg./kg. of body weight are generally used clinically.

TABLE 10.—*Bicarbonate Ion Concentration After Subconjunctival Injection of Acetazoleamide, 20 Mg. in 0.2 Ml.**

	Plasma		Aqueous Humor		Ratio	
	A. Time, 30 Min.					
	0 Min.	30 Min.	0 Min.	30 Min.	0 Min.	30 Min.
	15.1	25.2	28.8	29.8	1.91	1.18
	16.1	22.8	35.8	31.2	2.22	1.37
	9.6	18.1	31.6	26.9	3.30	1.48
	13.2	20.9	29.6	28.0	2.24	1.34
	13.3	22.8	28.9	27.6	2.17	1.21
	14.3	17.5	32.8	30.2	2.29	1.72
Av.	13.6	21.1	31.3	29.0	2.36	1.38
S. D.	2.1	2.8	2.5	1.5	0.42	0.20
t	3.676		1.917		4.900	
	B. Time, 24 Hr.					
	0 Min.	24 Hr.	0 Min.	24 Hr.	0 Min.	24 Hr.
	18.4	22.0	31.0	33.0	1.68	1.50
	21.3	22.0	31.0	31.5	1.35	1.43
	22.5	13.8	29.0	26.8	1.29	1.94
	15.8	12.9	28.5	30.6	1.81	2.37
	17.2	20.7	26.1	27.3	1.52	1.32
	19.4	24.1	30.1	33.4	1.55	1.39
Av.	19.1	19.3	29.3	30.5	1.53	1.66
S. D.	2.3	4.3	1.8	2.7	0.20	0.20
t	0.09		0.923		2.412	

* Values expressed as millimoles per liter.

level of the aqueous humor occurred 60 minutes after the subconjunctival injection of 10 mg. of acetazoleamide (Table 8). No significant lowering of the bicarbonate ion concentration occurred in 30 or 180 minutes (Tables 7 and 9). That this lowering of the bicarbonate ion concentration in the aqueous humor is probably not associated with the direct physiologic action of acetazoleamide is suggested by the data in Tables 10 and 11, which show that higher doses, 20 mg. of the drug, did not appreciably affect the bicarbonate ion concentration of the aqueous humor in 30 or 60 minutes.

During the course of this investigation it was observed that frequently the aqueous humor that was removed from an eye given subconjunctival injection of acetazoleamide became a gel on standing. Analysis of the fluid portion arising by agitation of the gel with a glass rod or pipette consistently gave lower bicarbonate values than in the control eye (Table 12). This phenomenon of gelation was frequently encountered during our studies with secondary or plasmoid aqueous humor and suggested that the subconjunctival injection of acetazoleamide increased the permeability of the blood-aqueous barrier. That this was the case was confirmed by protein analyses of the aqueous humor and by determining the fluorescein appearance time in the anterior chamber. The results, to be reported separately, show that the subconjunctival injection of 10 to 20 mg. of acetazoleamide in 0.2 ml. caused in several animals a marked increase (up to 200%) in the protein concentration of the aqueous humor in 30 and 60 minutes; time of appearance of fluorescein in the anterior chamber was decreased about 50%. It is therefore, reasonable to conclude that the subconjunctival injection of acetazoleamide effectively increases the permeability of the blood-aqueous barrier, thereby permitting the entrance of blood plasma into the aqueous

TABLE 11.—*Bicarbonate Ion Concentration After Subconjunctival Injection of Acetazoleamide, 20 Mg. in 0.2 Ml.**

	Aqueous Humor	
	0 Min.	60 Min.
	22.2	30.0
	24.6	31.6
	32.4	29.8
	30.0	28.8
	24.1	19.8
	34.8	28.8
	29.2	27.6
	26.6	25.0
	27.2	23.4
Av.	27.9	27.1
S. D.	3.8	3.6
t	0.500	

* Time, 60 minutes; values expressed as millimoles per liter.

TABLE 12.—*Bicarbonate Ion Concentration of Aqueous Humor, Which Gelled on Standing, After Subconjunctival Injection of 20 Mg. of Acetazoleamide in 0.2 Ml.**

O. S.	O. D.	Diff.
34.4	30.1	—4.3
32.6	23.1	—9.5
36.6	21.0	—15.6
31.3	24.0	—6.9
34.6	27.0	—7.6
39.2	31.1	—8.1
36.6	22.9	—13.7
41.0	34.9	—6.1
35.8	26.8	—9.0

* O. S., control eye; O. D., injected eye. Values are expressed as millimoles per liter. Time of tapping right eye, 60 minutes after injection.

humor.¶ Since the bicarbonate ion concentration of the blood plasma is about 10 mM/liter # less than that in the anterior aqueous humor, the observed fall in bicarbonate ion concentration in the latter could easily be caused by such a dilution by diffusion. In those experiments in which such a drop was not observed, it is assumed that the contribution of the blood plasma to the aqueous humor at the given time was insufficient to affect the bicarbonate ion concentration appreciably.

B. Blood Plasma: The results in Tables 6 to 11 show that the subconjunctival injection of 5 to 20 mg. of acetazoleamide did not significantly lower the bicarbonate ion concention in the blood plasma. The indicated increase (7.5 mM/liter) in blood bicarbonate (Table 10) may have been associated with the temporary low original concentration (13.6 mM/liter), since the same animals 24 hours later had a blood value of 19.1 mM/liter. The diurnal fluctuations of the bicarbonate ion concentration in the blood plasma over a period of three hours prevent any assignment of significance to the indicated rise (5.7 mM/liter) after 180 minutes (Table 9).

COMMENT

Although the mechanism whereby acetazoleamide reduces intraocular pressure in glaucomatous eyes is still unknown, these studies may permit a better understanding of ocular physiology and dynamics of aqueous humor formation. The results of this investigation tend to make untenable the heretofore reasonable assumption that the carbonic anhydrase activity of the anterior uvea mediated the elaboration or active transfer of bicarbonate ions into the aqueous humor of the rabbit eye.

¶ This may be the reason why the subconjunctival administration of acetazoleamide is not clinically effective in reducing the intraocular pressure of glaucomatous eyes.

The accumulated determinations of bicarbonate ion concentration (mM/liter) in blood plasma and anterior aqueous humor of 95 animals gave the following data: blood: 19.4, S.D. 5.3; aqueous humor: 30.1, S.D. 3.6; ratio: 1.55, S.D. 0.37.

SUMMARY

The determination of the bicarbonate ion concentration of 95 animals by the Warburg technique gave the following values, expressed in millimoles per liter: blood, average 19.4 ± 10.6; anterior aqueous humor, 30.1 ± 7.2; ratio, 1.55 ± 0.74. The range is determined by 95% confidence limits expressed as 2 standard deviations. Intravenous and subconjunctival injection of acetazoleamide (Diamox) did not appreciably lower the bicarbonate ion concentration in the anterior aqueous humor or in the blood plasma of a normal rabbit. It is concluded that the carbonic anhydrase activity of the anterior uvea is not necessary for the elaboration or maintenance of bicarbonate ion concentration in the anterior aqueous humor of the normal rabbit eye.

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Determination of Acetazoleamide in Aqueous Humor of the Rabbit Eye

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In two previous papers * it had been shown that the intravenous and subconjunctival injection of aceatzoleamide (Diamox) completely inhibited the carbonic anhydrase activity of the anterior uvea of the rabbit eye, without appreciably lowering either the normal intraocular tension or the concentration of bicarbonate ion in the aqueous humor.

A better understanding of the mechanism of action of acetazoleamide in the management of glaucoma depends upon a knowledge of the medium through which the drug functions. It was of interest, therefore, to determine whether the drug entered the aqueous humor of the eye after local and systemic administration, and in what concentration.

The only method available for the determination of acetazoleamide in biological fluids was that of Maren, Ash, and Bailey.† It is based essentially upon the changing pH method of Philpot and Philpot [4] and is dependent on the rate of hydration of CO_2 in the presence of the carbonic anhydrase inhibitor. Despite the reported advantages of high sensitivity, accuracy, and specificity, the method is indirect and requires a special set-up under carefully controlled conditions.

From the Wills Eye Hospital, Department of Research.

* References 1 and 2.

† Prior to the initiation of this study, Dr. Maren made available to us a preprint of the paper, which at that time did not include his preliminary investigations of a spectrophotometric method.

This report describes a simple and direct spectrophotometric method for the determination of acetazoleamide in the aqueous humor of the eye. Measurable quantities of the drug were found in the anterior chamber after local and systemic administration.

MATERIALS

The sodium salt of acetazoleamide was supplied by Dr. James Gallagher, of Lederle Laboratories. Reagent grade absolute methanol was obtained from the Baker Chemical Co. All optical densities were measured in microcuvettes with a Beckman Spectrophotometer, Model DU.

Pigmented rabbits, 5½ to 6½ lb. (2.5 to 2.9 kg.), were used. The eyes were anesthetized with tetracaine hydrochloride and then thoroughly flushed with isotonic saline solution. The aqueous humor was removed through a 26-gauge needle into a tuberculin syringe and emptied into a conical tube, from which the desired aliquot was removed.

EXPERIMENTAL PROCEDURE

The ultraviolet absorption spectrum of an aqueous solution of acetazoleamide in concentrations up to 50γ/ml., pH 6.5 to 8.9, possessed a maximum peak at 265 mμ. When the pH was adjusted to 10 or higher, the position of the maximum peak shifted to 290 mμ, as shown in Figure 1. The optical density of the peak at 290 mμ is about 70% larger than that of the peak at 265 mμ. The absorption at 290 mμ follows Beer's law for concentrations as high as 50γ/ml. The optical density per microgram of acetazoleamide was 0.05.

In order to utilize the absorption characteristics of acetazoleamide as a basis for its quantitative determination, it was necessary to remove selectively the substances in aqueous humor that were responsible for its relatively high absorption in the ultraviolet

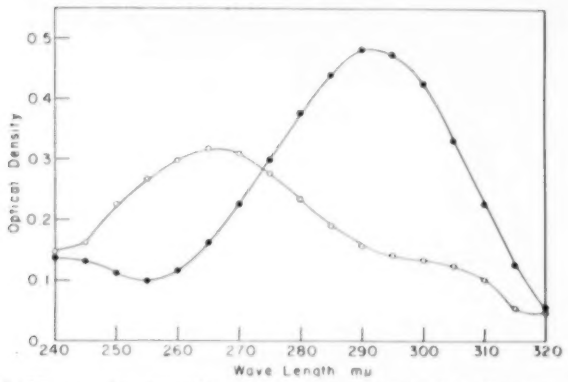

Fig. 1.—Effect of pH upon the absorption spectrum of acetazoleamide. The line of clear circles indicates values for 10γ of acetazoleamide in 1 ml. of H_2O; the line of black circles, the values for 10γ acetzoleamide in 1 ml. of 0.1 N NaOH.

spectrum. The two major components that contribute largely to the ultraviolet absorption properties of aqueous humor are proteins and ascorbic acid. The latter, present in concentrations of 28 mg. per 100 cc. in rabbit aqueous, possesses a maximum absorption peak at 265 mμ in solutions of pH from 5 to 10. Above pH 10, however, this peak is almost completely destroyed, and the ultraviolet spectrum is practically zero for original concentrations of ascorbic acid of 50γ/ml. The contribution of the protein fraction to the over-all absorption in the region of 230 to 320 mμ could be reduced to a minimum by heating a 1:10 dilution of aqueous humor at 50 C for 10 minutes and removing the precipitate formed. Under these conditions the absorption spectrum of aqueous solutions of acetazoleamide was substantially unchanged. Attempts to reduce the absorption of normal rabbit aqueous humor at 290 mμ to 0 or a negligible value failed.‡ It was necessary, therefore, to establish a control value for normal aqueous humor.§ In all experiments, accordingly, the normal aqueous humor was removed from one eye of the animal prior to systemic or local administration of the drug, and the aqueous humor of the other eye was used for experimental evaluation.

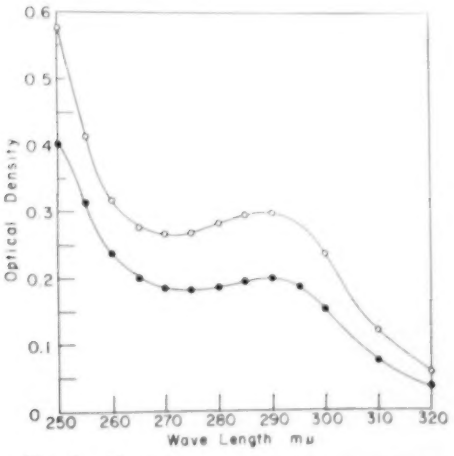

Fig. 2.—Absorption spectrum of aqueous humor with and without acetazoleamide. In both cases the aqueous humor (0.15 ml.) was treated as described in the text. Line of black circles indicates normal aqueous humor; the line of clear circles, aqueous humor from an eye that had been injected subconjunctivally with acetazoleamide.

The analytical procedure devised for the quantitative determination of acetazoleamide in the aqueous humor of the eye is as follows: Aqueous humor, 0.1 to 0.2 ml., was pipetted into a glass-stoppered conical centrifuge tube containing 2.0 ml. of absolute methanol. (Acetazoleamide is more soluble in methanol than in water.) The aqueous methanol solution was heated in a water bath at 50 C for 10 minutes. The denatured proteins were

‡ Hexane extraction of the methanol solution of aqueous humor, to remove lipids, had no effect on the absorption spectrum.

§ An average optical density of 0.196/0.15 ml. of aqueous humor was obtained.

separated by centrifugation; the supernatant was poured off and the residue washed with 90% methanol. The methanol was removed under reduced pressure at a bath temperature of 45 C and the resulting residue dissolved in 1.0 ml. of 0.1 N NaOH. The optical density of the caustic solution was measured at 290 mμ against a 0.1 N. NaOH solution used as a blank.

A typical experimental observation is given in Figure 2, which shows the absorption spectrum of normal aqueous humor and of aqueous humor from an eye that had received subconjunctival injections of 10 mg. of acetazoleamide. The addition of 1γ to 20γ of acetazoleamide to 0.15 ml. of aqueous humor (2.7 to 54.0×10^{-5} M) could be recovered quantitatively by the above-described procedure.

RESULTS

Fifteen to 60 minutes after intravenous injection of 30 mg. of acetazoleamide per kilogram of body weight, concentrations as high as 4.6γ per 0.2 ml. of aqueous humor (9.4×10^{-5} M) were found; 30 to 90 minutes after subconjunctival injection of 5 to 10 mg. of the drug, concentrations from 3γ to 38γ per 0.2 ml. of aqueous humor (6.2×10^{-5} M to 7.8×10^{-4} M) were found.‖

The above-described method was not applicable to the determination of acetazoleamide in extracts of ciliary body and iris because of the presence of interfering absorbing material that could not be removed. However, analysis of the tissue extracts by enzyme inhibition studies indicated the presence of 10γ to 15γ of acetazoleamide in an extract equivalent to one ciliary body-iris tissue after subconjunctival injection and from 5γ to 8γ after intravenous injection.

‖ After this paper had been prepared for publication, we learned of the unpublished observations of Ballintine and Maren[3] (cited in reference 3), who found in the aqueous humor of rabbit 0.6γ and 0.5γ per milliliter, respectively, 2 and 0.5 hours after intravenous administration of acetazoleamide (25, and 10 mg/kg., respectively).

SUMMARY

A microspectrophotometric method for the determination of acetazoleamide (Diamox) in the aqueous humor of the rabbit eye is described.

Concentrations up to 4.6γ per 0.2 ml. of aqueous humor were found after intravenous injection of 30 mg. of the drug per kilogram of body weight, and from 3γ to 38γ per 0.2 ml. of aqueous humor was found after subconjunctival injection of 5 to 10 mg. of the drug.

By means of enzyme inhibition studies with extracts of ciliary body-iris tissue, concentrations from 10γ to 15γ of acetazoleamide were found in one tissue after subconjunctival injection and from 5γ to 8γ after intravenous injection.

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New Method for Fractionation of Lens Proteins

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In 1894 Morner demonstrated that there exist four different holoprotein fractions in the lens. A fraction insoluble at physiological pH was called albuminoid. Three water-soluble fractions were found: two atypical globulins (α and β crystallin) and an albumin-like fraction (γ crystallin).

The separation and elimination of the insoluble fraction from the crude extract is simple and made by centrifugation. The separation and fractionation of the water-soluble fractions is a much more complicated problem. Following Morner, different authors have tried to establish a suitable fractionation device, mostly without any control (Krause, 1932; Woods and Burky, 1927; Burky and Woods, 1928; Bonot and Nordmann, 1946; Nordmann, 1949). On the other hand, the proteins by these methods of fractionation are often profoundly modified and even largely denaturated by the different manipulations. The aim of this work is to obtain well-defined and homogeneous protein fractions from the point of view of electrophoretic control.

Ox lenses were chosen as material because of the relatively large quantity of protein available (concentration $\pm 5\%$).

Aqueous extracts of lens proteins are prepared by grinding fresh decapsulated ox lenses (cortical or nuclear part) and subsequently adding distilled water. Centrifugation at 15,000 rpm for 45 minutes is carried out in order to remove the insoluble albuminoid. As we have previously demonstrated (1953), the pherogram (paper electrophoresis technique of Grassmann and Hannig; buffer solution pH 8.6; 0.1μ) of the ox lenticular proteins reveals three water-soluble fractions: Fraction I, the most mobile (32%); Fraction II (53%), and Fraction III (15%). The concentration of these three fractions is different in the nucleus and in the cortex. Fraction III, the least mobile fraction, predominates markedly in the nuclear portion.

Experience has shown us that Fraction I may be obtained in a pure state; Fractions II and III are, on the contrary, highly resistant to further separation, even when the pH, the temperature, and the dielectric constant of the solvent are very carefully controlled.

TABLE 1.—*Protein Fractions in the Ox Lens*

Fraction	Cortex, %	Nucleus, %
I	39.1	25.6
II	56.5	35.3
III	4.5	39.0

As the aqueous extracts of the cortex and of the nucleus behave in a very different fashion, we shall describe separately the techniques which are applicable to each.

I. FRACTIONATION OF THE "CORTEX" PROTEINS

1. *Separation of a_1c and a_2c Crystallin.*—The aqueous extract of the lens cortex of oxen is shown by electrophoresis to consist of three protein fractions (Fig. 1). The aqueous extract is first brought to pH 4.3 with HCl 1 N/10 and thereafter to pH 5.0 with NaOH N/10. A precipitate is formed which mainly consists, as shown by electrophoresis, of Fraction I (Fig. 2). This fraction is called a_1c crystallin.

Researcher of Belgian National Fund for Scientific Research (Dr. Rabaey).

Professor of Ophthalomolgy, University of Ghent (Dr. François).

From the Ophthalmological Clinic of the University of Ghent; Director, Prof. J. François, M. D.

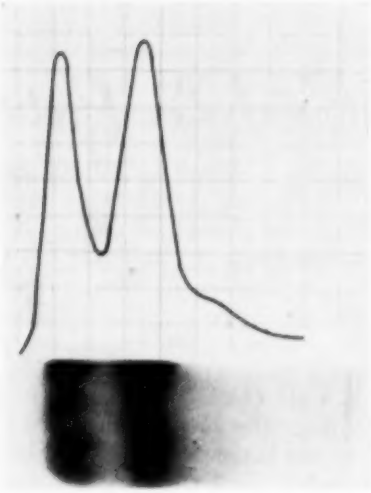
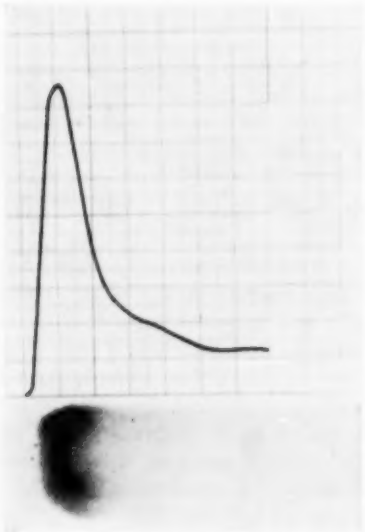


Figure 1

The α_1c crystallin is eliminated by centrifugation and dissolved in barbital sodium (Veronal sodium) buffer (Michaelis) or phosphate buffer (pH 8).

The yield of this α_1c is very weak; the major part of Fraction I remains in the solution, as is shown by a pherogram obtained from the supernatant fluid (Fig. 3).

Figure 2



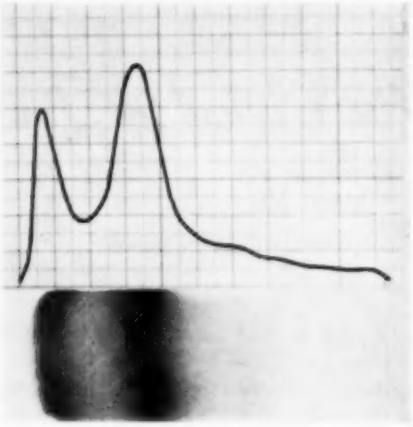
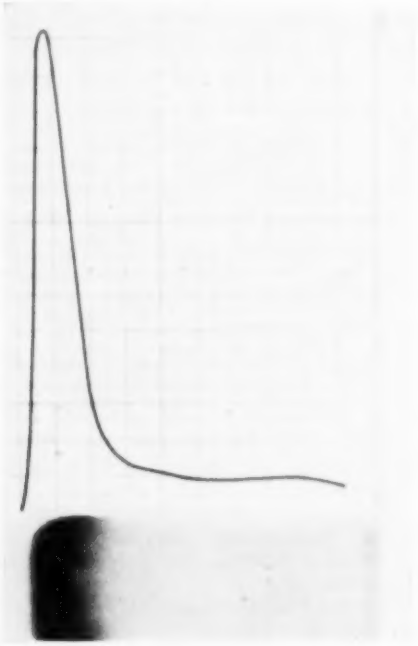


Figure 3

To the supernatant fluid, at a temperature between 5 C and 10 C, ethyl alcohol is added slowly while stirring until a concentration of 13% is reached. A new precipitate is formed, which also consists almost exclusively of Fraction I (Fig. 4). This fraction we called α_2c crystallin. This precipitate is centrifugated and dissolved like α_1c crystallin.

Figure 4



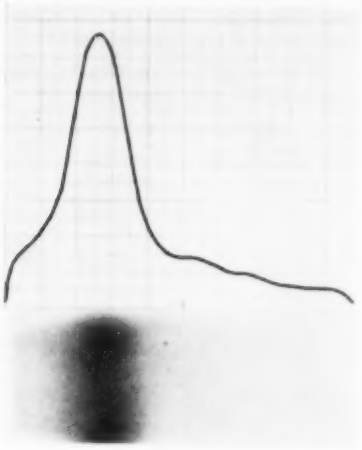
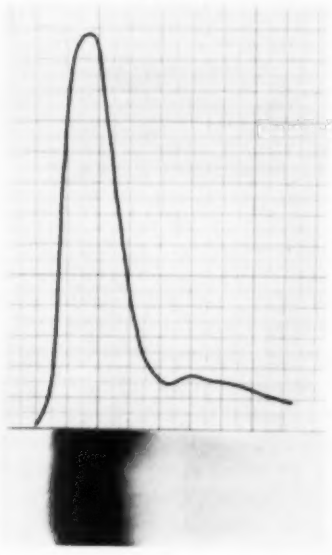


Figure 5

Although the major part of the Fraction I is now eliminated from the aqueous extract (90% to 98%), there remains nevertheless variable portions of Fraction I in the supernatant fluid (Fig. 5). This is removed by increasing, at the same temperature, the concentration of alcohol to about 17%. The precipitate now obtained is no longer homogeneous, but this operation is indispensable for eliminating Fraction I completely from the solution.

Figure 6



2. *Separation of the βc Crystallin.*—The supernatant fluid is rendered alkaline (pH 8) with NaOH N/10 or with barbital sodium buffer. Sodium chloride 0.5% is also added, after which at a temperature below 0 C the concentration of alcohol is further increased to 26%. When the temperature is lowered to about —10 C a precipitate forms, which is recovered by filtration, still at the same low temperature. The precipitate is redissolved at a temperature below 0 C in buffer solution.

Figure 7

Thus is obtained a fraction which we call βc crystallin. The pherogram of this βc crystallin shows the peaks of the Fractions II and III from the cortical lens protein (Fig. 6). Only a small quantity of Fraction III is present.

II. FRACTIONATION OF THE "NUCLEUS" PROTEINS

1. *Separation of the αn Crystallin.*—The aqueous extract of the nucleus, containing

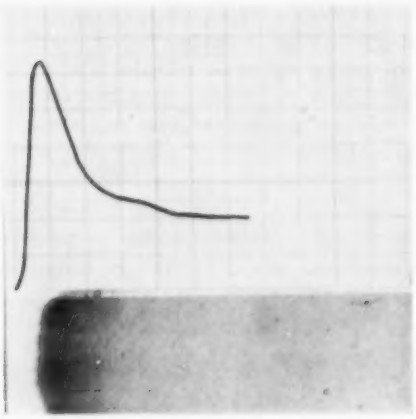
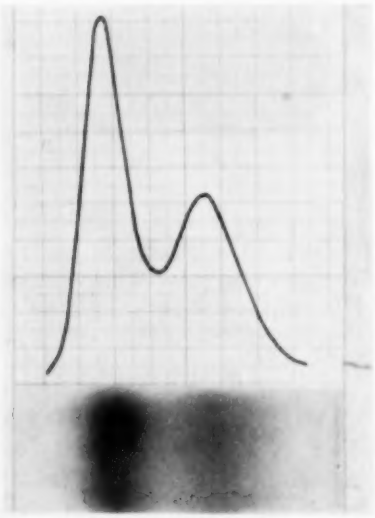


Figure 8

the three electrophoretic fractions already known (Fig. 7), is first brought to pH 4.5 by adding HCl N/10, then brought to pH 5.0 by the addition of NaOH N/10. A bulky precipitate is obtained, which is recovered by filtration, and the precipitate is dissolved as usual. This fraction is called αn crystallin. This αn crystallin consists almost exclusively of the nuclear Fraction I (electrophoretic control) and contains only a few traces of Fractions II and III (Fig. 8). This Fraction

Figure 9



I can be obtained in an even purer state by redissolving and reprecipitation.

2. *Separation of the βn Crystallin.*—The filtrate obtained after elimination of the αn crystallin contains generally only a small per cent of Fraction I (Fig. 9). The pH is brought to 8.0 and at a temperature of —4 C. Sodium chloride 0.5% and ethyl alcohol to

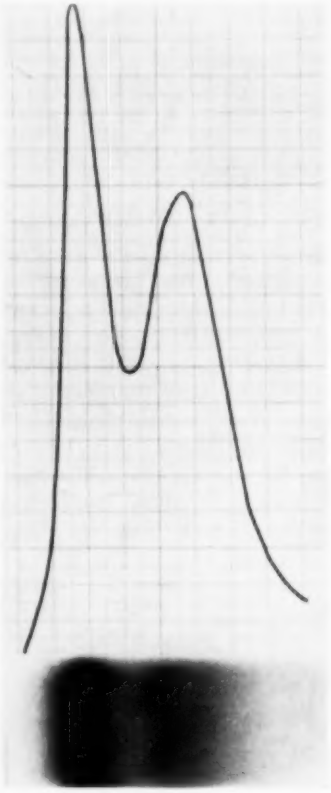


Figure 10

a concentration of 13% are added. The precipitate which is obtained under these conditions is recovered by filtration at the same temperature and redissolved as usual below 0 C.

This fraction is called βn crystallin. By electrophoresis no trace of αn crystallin is found, but two peaks of variable height corresponding to the nuclear Fractions II and III appear (Fig. 10).

Freeze-Drying.—The different protein portions separated by our method have been dried by the freeze-drying technique. We thus obtained completely soluble white powders. The pherograms of the redissolved proteins are identical with those obtained before the drying.

COMMENT

A fact emerging immediately from these experiments is that the fractionation of the water-soluble proteins of the cortex is much more difficult than that for the proteins of the nucleus. The αn crystallin is obtained more easily than the αc crystallin. By electrophoresis, on the other hand, clear differences are observed. Even if the mobility of these α fractions is analogous, a considerable amount of the αn crystallin is absorbed on the paper while migrating on the paper strip.

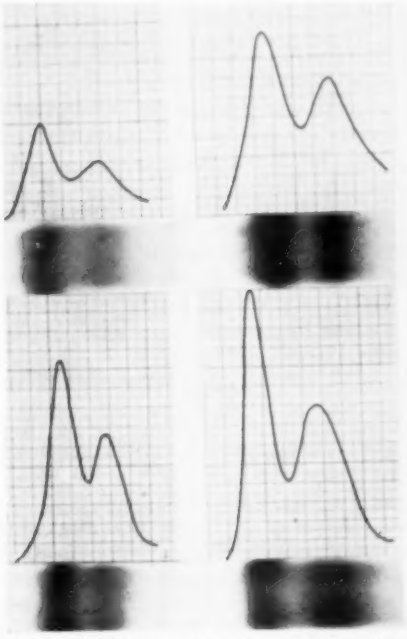


Fig. 11.—Effect of pH's 5.2, 6.5, 7.0, and 8.0 on the βn crystallin in the presence of 21.5% alcohol.

Attempts to fractionate βn crystallin in order to obtain the electrophoretic Fractions II and III have proved unfruitful. Table 2 demonstrates this point: It sums up the influence of the pH and concentration of alcohol (temperature 4 C) on the fractionation of βn crystallin. Although the amounts of Fraction II and Fraction III in the precipitate vary from 40% to 60% for a concentration of 18.4% alcohol, no clear separation, however, could be obtained (Fig. 11).

TABLE 2.—*Effect of Alcohol Concentration and pH on Fractionation of βn Crystallin*

Concentration of Alcohol, %	pH 5.2		pH 6.15		pH 7		pH 8	
	II	III	II	III	II	III	II	III
18.4	42	58	61	39	60	40	40.5	59.5
21.5	57	43	52.5	47.5	56.5	43.5	49.5	40.5

The value of our method of fractionation lies in the fact that we are able to obtain protein fractions which are clearly defined and in sufficient quantities to permit chemical or physical study.

Thus, we have already been able to make several determinations of molecular weight (M. W.), by light scattering.*

Fig. 12.—Lens proteins in barbital sodium buffer diluted. Reduced to 52% of mag. × 30,000. University of Ghent Center of Electron Microscopy.

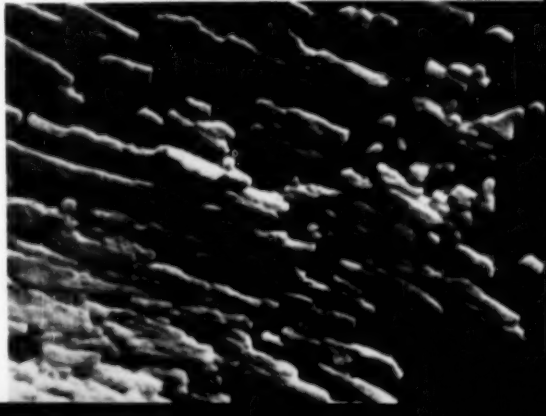


Fig. 13.—Lens proteins in barbital sodium buffer diluted. Reduced to 52% of mag. × 30,000. University of Ghent Center of Electron Microscopy.

The intensity of the scattered light was measured over a wide variety of angles, and the values have been plotted according to Zinn's method. Calibration was made with pure toluene. The results of this investigation seem to indicate that the molecular weight of the Fraction I (α crystallin) is much higher than those of Fractions II and III (β crystallin). For instance, the fraction βc has given a M. W. to the order of 200,000 and the α_2c a M. W. to the order of 1,200,000.

It also became evident that the molecular weight of the lens proteins increases markedly in the nuclear part of the lens.

These same "molecules" or "particles" become so large that, although they remain completely soluble and move in the electric field, they are easily seen with the electron microscope.

These pictures, taken with the electron microscope (G. Vandermeerssche, Center of Electron Microscopy in Ghent), for the existence of particles with a diameter of about 400 A. (Fig. 12).

These quasispherical particles have a marked tendency to gather together in more complex formations, of which Figure 13 gives an example: The side-by-side orientation sketches a design of fibers.

SUMMARY

A new method of fractionation, inspired by the work of Cohn and his school, permits the obtention of simple protein fractions from the aqueous extract of ox lenses. As there exists a very clear difference between the cortex and the nucleus, we distinguish for the cortex the α_1c and α_2c and βc crystallin and for the nucleus the αn and βn crystallin. Although the α crystallins are electrophoretically homogeneous, the β crystallins are, on the contrary, resolved by paper electrophoresis into two peaks.

Physical study reveals the existence of very large molecules, especially in the nucleus, which possess an evident tendency to agglomeration, of which the degree must be of great importance in the aging of the lens and formation of cataract.

* Professor Sadron, Director of the Centre for the studies of macromolecular physics at Strasbourg, cooperated in this study, and MM. C. Wippler and A. J. Hyde collaborated.

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Studies on Experimental Glaucoma

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and
IRVING H. LEOPOLD, M.D., Philadelphia

The aim of the present investigation is the study of experimentally induced glaucoma in the rabbit and the influence of iridectomy on its course. The normal intraocular pressure can be raised (*a*) by decreasing the aqueous drainage or (*b*) by increasing the rate of fluid production.

1. Diminished aqueous outflow has been achieved by mechanical blockage of the filtration angle. This can be accomplished in the following ways: (*a*) by injecting oils and colloids that hinder the outflow of aqueous at the angle of the anterior chamber [1]; (*b*) by producing an inflammatory response at the angle, with ultimate development of synechiae [1]; (*c*) by developing a proliferative endothelial reaction at the angle by means of electrolytic products,[1] and (*d*) by occlusion of the pupil, which also leads to mechanical blockage of the angle of the anterior chamber. Interruption of the aqueous circulation at the pupil has been produced by obstructing the pupillary aperture with air or solid discs. Scheie and Frayer [2] and Barkan [3] induced air glaucoma, by which fluid in the posterior chamber is trapped and pushes the root of the iris forward until the angle is obstructed. Wortham,[4] by inserting a grooved plastic disc into the pupil, was able to precipitate an acute glaucoma and iris bombé, which disappeared when the iris separated from the disc.

2. An increase in the aqueous inflow can be produced by substances that, applied locally, augment the caliber and permeability of the ocular vessels. Vascular glaucomas of this nature have been produced by histamine, a nitrogen mustard, and isofluorophate (DFP). Friedenwald and Pierce [5] were able to produce an acute increase in pressure by injecting histamine into the eyes of dogs. At the same time, they observed a narrowing of the anterior chamber and the bombé appearance of the iris, which subsided when a stream of fluid passed from the posterior to the anterior chamber, with a simultaneous decrease in tension. Davson and associates * have demonstrated the profound influence that vascular and neurological factors exert on the severe congestion and the acute hypertension due to nitrogen mustard. Von Sallmann and Dillon [8] held the opinion that the rise in ocular tension following instillation of isofluorophate is caused by increased permeability of the ciliary capillaries, and in their experiments treated and control eyes did not show a constant difference in the depth of the anterior chamber.

For descriptive purposes, the investigations presently reported are divided into two sections. The first is concerned with the study of the configuration of the anterior chamber, and the second refers to the evaluation of the action of two types of antiglaucomatous operations (peripheral and complete iridectomies).

A. CONFIGURATION OF THE ANTERIOR CHAMBER

The configuration of the anterior chamber has been believed to play an important part in the etiology of glaucoma since Knies,[9] in 1876, described in glaucomatous eyes the presence of peripheral adhesions between the iris and the cornea. On the basis of this observation, he pointed out that the cause

From the Research Department of Wills Eye Hospital.

* References 6 and 7.

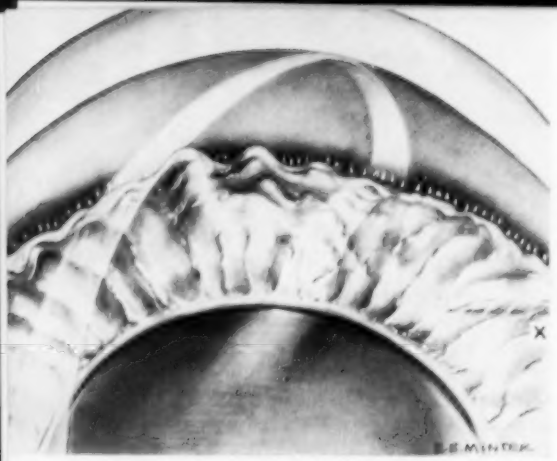
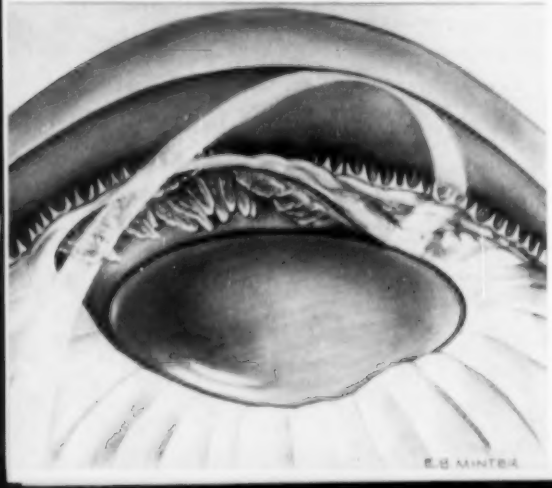


Fig. 1.—Anterior chamber angle in normal albino rabbit. At *X* needle inserted into the posterior chamber is visualized behind the iris. Reduced 71% from mag. × 12.

of increased intraocular pressure is an obstruction to the normal outflow of the aqueous humor. Weber [10] and Priestley Smith [11] supported similar pathogenetic views, differing only on the explanation of the mechanism by which the angle of the anterior chamber becomes occluded.

The modern mechanical theory of the origin of glaucoma started when Raeder,[12] in 1923, demonstrated that acute congestive glaucoma is associated with a shallow anterior chamber, whereas the chronic simple type is more frequently associated with a deeper chamber. With the development of gonioscopy, ample evidence was accumulated in favor of the obstructive theory. Barkan,[13] in 1938, introduced a classification based on the appearance of the angle of the anterior chamber as observed with the gonioscope. Notwithstanding the progress accomplished, final proof must wait until all the factors involved and their mechanism of action are identified.

Fig. 2.—Appearance of the anterior chamber angle in an albino rabbit after complete iridectomy. Reduced 71% from × 11.



The factors that determine the depth of the anterior chamber have been divided by Sugar [14] into two groups, anatomical and physiological. In the opinion of the mechanical school, the basic anatomical factors are refractive errors, lens growth, corneal size, and iris thickness. The physiological factors, also known as angle-crowding factors, include accommodation, pupillary dilatation, ciliary congestion, and relative pupillary block with physiological iris bombé.[15]

Method

The study of the morphology of the chamber angle was accomplished in vivo by gonioscopic observation and in vitro by histological section.

Between 5 and 10 albino rabbits were used for each group of observations. The animals weighed between 3 and 6 lb. (1.3 and 2.7 kg.). They were anesthetized with thiopental (Pentobarbital †) sodium (0.2 ml./lb., or 0.4 ml./kg.) and placed in a lateral position for gonioscopic examination. In some cases a canthotomy was performed, and after slight proptosis of the eye the contact glass was applied. The liquid used for injection under the lens was methylcellulose 1%.

Histological sections were made according to the cedar oil-celloidon method and stained with hematoxylin and eosin.

After becoming familiar with the normal appearance of the angle,[16] we modified the configuration of the anterior chamber by mechanical procedures and the use of drugs.

I. The alterations induced by physical agents consisted in the following:

(*a*) Removal of the aqueous, followed by injection of air or isotonic saline into the anterior chamber.

(*b*) Introduction of isotonic saline or air through the posterior chamber. This was achieved by the insertion across the ciliary body of a bent needle whose position was observed through the iris (Fig. 1).

(c) Iris surgery consisting in peripheral and complete iridectomies. In eyes that had been operated on observations were made two to four weeks after surgery had been performed.

† Pentobarbital sodium, supplied as a sterile solution containing 60 mg. per milliliter.

II. The following pharmacological effects served as a basis for the selection of drugs:

(*a*) Miosis, induced by the use of physostigmine salicylate, 2%, instilled in the conjunctiva.

(*b*) Mydriasis, produced with atropine sulfate, 1%, used by the same route.

(*c*) Glaucomatous agents, including isofluorophate, 10%, and a nitrogen mustard,‡ 0.5%, which were applied as a conjunctival instillation of 0.05 ml. The eyes were removed for histological sections when the hypertensive effect of these drugs was at its peak.

Results

Gonioscopic findings in this experiment can be grouped in the following classes:

I. Alterations in the configuration of the anterior chamber produced by mechanical agents.

(*a*) Changes induced through the posterior chamber.

1. By injection of air into the posterior chamber, we were able to produce occasionally a total, but more frequently only a circumscribed, iris bombé accompanied by closure of the angle. These changes were more easily induced when miosis was present.

2. When saline was injected, only transitory bulging of the iris was noticed in the area surrounding the point of the needle (Fig. 1). This occurred independently of the pupillary size. When great pressure was applied, cloudiness of the cornea developed, which interfered with further observation.

(*b*) Changes induced through iris surgery.

In eyes with complete and peripheral iridectomies a slight widening of the aperture of the angle was present. This widening was more marked in the area surrounding the region of operation and was independent of the appearance of the surgical scar of the iris (Fig. 2).

(*c*) Changes induced through the anterior chamber.

1. Injection of air into the anterior chamber after withdrawal of the aqueous humor produced a depression of the peripheral third of the iris, which became markedly concave anteriorly (this area is convex in the normal eye). The angle was widely open, and the central two-thirds of the iris was pressed against the anterior surface of the lens and had a convex appearance (Fig. 3). This effect could be obtained only when the intraocular tension after the injection of air was well above normal. Similar results have been reported by Hughes and Cole.[17]

2. No gonioscopic observations could be made with injection of fluid because rapid clouding of the cornea occurred when the pressure was increased. Friedenwald and Pierce[18] were unable to note any change in the depth of the anterior chamber during intracameral saline injections.

II. Modifications produced by pharmacological agents.

(*a*) With mydriatic and miotic drugs, changes in the aperture of the angle are difficult to induce. Alterations were observed only in cases of maximal dilatation or contraction. The former produces slight narrowing, and the latter widening, of the angle.

(*b*) With nitrogen mustard and isofluorophate, one could not observe a physiological iris bombé with closure of the angle, owing to partial pupillary block. In general, with isofluorophate the width of the angle visualized appeared proportional to the degree of miosis. Observations with nitrogen mustard were less satisfactory because of the development of cloudiness. This drug causes a marked congestion of the superficial vessels of the iris, which counterbalances the angular widening produced by the miosis.

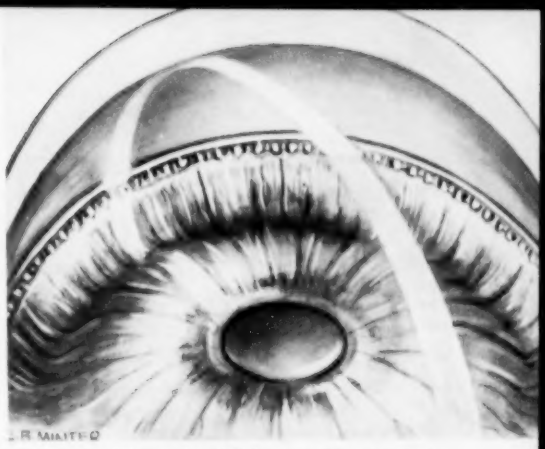


Fig 3.—View of rabbit's anterior chamber after substitution of the aqueous humor by air.

‡ Mechlorethamine hydrochloride, also known as methyl-bis(β-chloroethyl) amine hydrochloride, in vials containing 10 mg. of the drug in dry form triturated with 90 mg. of anhydrous sodium chloride.

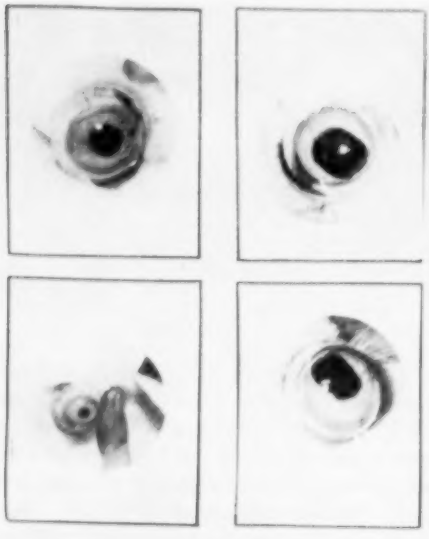
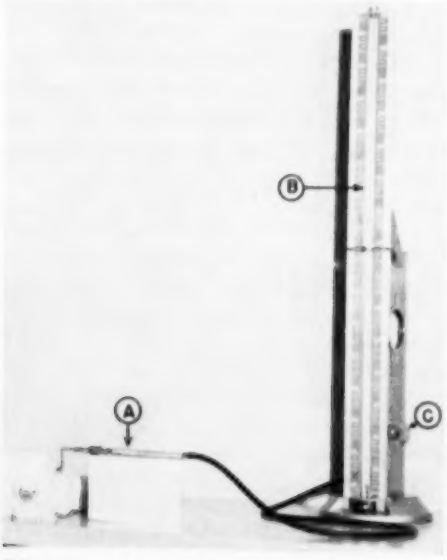


Fig. 4.—Photographs illustrating the average type of peripheral and complete iridectomies performed in the rabbit eyes.

(*c*) In eyes operated on the modifications of the angle previously described were not significantly altered by the drugs employed.

Fig. 5.—General view of phlebomanometer: *A*, observation system; *B*, scale of manometer; *C*, control knob of compensatory mechanism.



Measurements of the angle in histological sections gave widely variable results, due to the difference in size of the eyes examined and the distortion produced by the process of sectioning. Notwithstanding this, the following qualitative observations could be made: In no case after the use of isofluorophate and nitrogen mustard was actual contact observed between the iris root and the cornea.

B. EFFECT OF PERIPHERAL AND COMPLETE IRIDECTOMIES IN EXPERIMENTAL GLAUCOMA

Basal iridectomy as a therapeutic procedure for the control of pressure in glaucoma was introduced by von Graefe [19] in 1857

Fig. 6.—Observation tube with two-way stopcock and bent needle.

Fig. 7.—Position of needle after insertion into the anterior chamber.

and at present is the surgical intervention of choice in acute cases.

The establishment of a communcation between the anterior and the posterior chamber by a small opening in the iris (peripheral iridotomy or iridectomy) was suggested by Curran § in 1920 as a measure for normalizing the tension in glaucomatous eyes. The

§ References 20 and 21.

indiscriminate use of this operation led early observers to believe that it produces only a temporary relief,[22] but in recent years it has been strongly advocated for the interval or preglaucomatous phase of narrow-angle glaucoma.[23]

The purpose of the present section is to determine the effect of these two types of operations in experimental glaucoma induced by a nitrogen mustard and isofluorophate.

Method

The intraocular pressure was measured in 250 rabbit eyes, which were divided into three groups: 100 normal eyes, 75 eyes with peripheral iridectomy, and 75 eyes with complete iridectomy. The animals

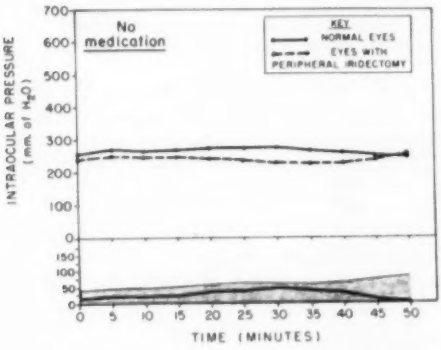


Fig. 8.—Intraocular pressure of normal eyes and eyes with peripheral iridectomy.

Lower graph: (1) Shaded area represents confidence region of the difference between means; significance level 1%. (2) Dark-line curve represents difference between means of the two curves in the upper portion of the graph; values falling within shaded area are not significant.

had a weight varying between 3 and 6 lb. and were anesthetized with thiopental sodium (0.2 ml./lb.), which was afterward supplemented by intraperitoneal injections of 0.5 to 1.5 ml. Profound narcosis was avoided because it suppressed the hypertensive ocular effect produced by nitrogen mustard.

Operations were performed under the usual sterile conditions. The ab externo technique was followed for the complete iridectomy. In the peripheral iridectomies special attention was given to the establishment of a definite communication between the anterior and the posterior chamber (Fig. 4). Between 10 and 14 days were allowed to elapse before the intraocular pressure was measured in the operative eyes.

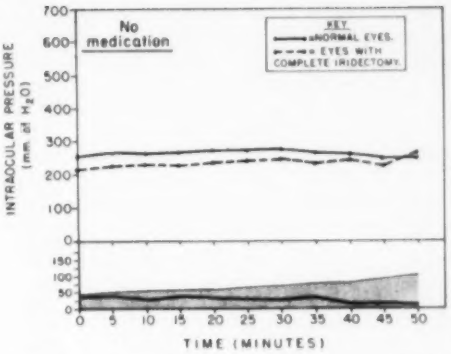


Fig. 9.—Intraocular pressure of normal eyes and eyes with complete iridectomy.

Lower graph: (1) Shaded area represents confidence region of the difference between means; significance level 1%. (2) Dark-line curve represents difference between means of the two curves in the upper portion of the graph; values falling within shaded area are not significant.

The drugs employed were a nitrogen mustard and isofluorophate. The former was prepared shortly before use and applied as a conjunctival instillation of 0.05 ml. of 0.5% aqueous solution in isotonic saline. Isofluorophate was used in a concentration of 10% in anhydrous peanut oil and the same amount instilled into the conjunctival cul-de-sac. Both agents were given 15 minutes after the insertion of the needle into the eye so that the pressure could settle to a reasonably constant level, to be used as a base line. Then readings were made

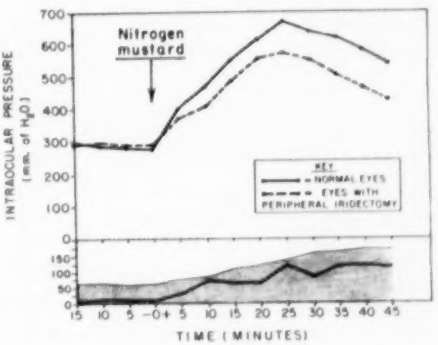


Fig. 10.—Effect of nitrogen mustard on the intraocular pressure of eyes not operated on and eyes with peripheral iridectomy.

Lower graph: (1) Shaded area represents confidence region of the difference between means; significance level 1%. (2) Dark-line curve represents difference between means of the two curves in the upper portion of the graph; values falling within shaded area are not significant.

every five minutes for as long as the animal remained calm under anesthesia. Each one of these substances was given to groups of 25 normal and 25 eyes operated on, and controls were observed that received no medication. The distribution of the experimental material is summarized in the table.

Distribution of Experimental Material

Eyes	Untreated	Nitrogen Mustard	Isofluorophate
Normal	50	25	25
With peripheral iridectomy	25	25	25
With basal iridectomy	25	25	25

The intraocular pressure was measured manometrically with a "phlebomanometer" (Fig. 5). The apparatus is a compensatory type of manometer and consists of three parts: an observation system with

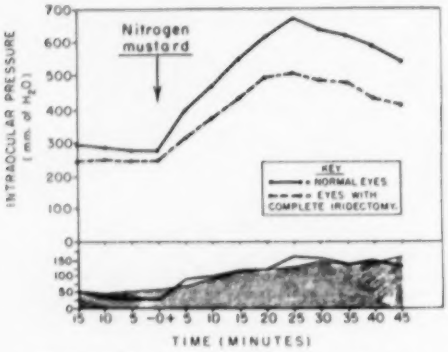


Fig. 11.—Effect of nitrogen mustard on the intraocular pressure of eyes not operated on and eyes with complete iridectomy.

Lower graph: (1) Shaded area represents confidence region of the difference between means; significance level 1%. (2) Dark-line curve represents difference between means of the two curves in the upper portion of the graph; values falling above shaded area are significant.

a needle to be inserted into the eye; a manometer to register the pressure, and, between the two, a compensatory mechanism that balances the inflow and outflow of fluids.

Before insertion of the needle the observation system is filled with an anticoagulant; in our case heparin ‖ was employed. The observation tube has a capillarity of 20 mm., which is compensated for by having the meniscus of the anticoagulant approximately 2 cm. vertically above the point where the needle is inserted. For this purpose, and in order to obtain a simpler manipulation, we used a

‖ Heparin sodium, 1000 U. S. P. units per milliliter in istonic sodium chloride solution.

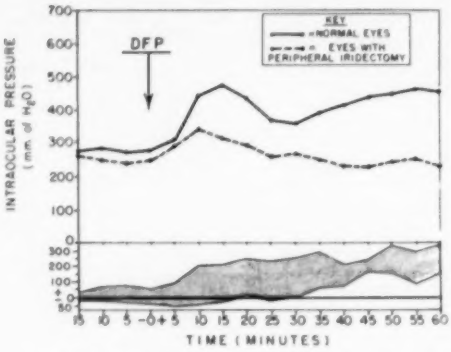


Fig. 12.—Effect of isofluorophate (DFP) on the intraocular pressure of eyes not operated on and eyes with peripheral iridectomy.

Lower graph: (1) Shaded area represents confidence region of the difference between medians; significance level 1%. (2) When this shaded area is entirely above the 0 line, the difference between medians is significant.

26-gauge needle bent twice (Fig. 6). The distal horizontal portion was inserted into the vertex of the cornea and remained in the anterior chamber without touching the iris. Between the needle and the observation tube a two-way stopcock was inserted. When the needle is thrust in the anterior chamber, this valve is closed to minimize any sudden pressure disturbance. A few minutes later it is opened, and communication between the needle and the observation tube is established (Fig. 7).

The manometer itself is of a water type and has a graduated scale attached that permits readings in millimeters of water.

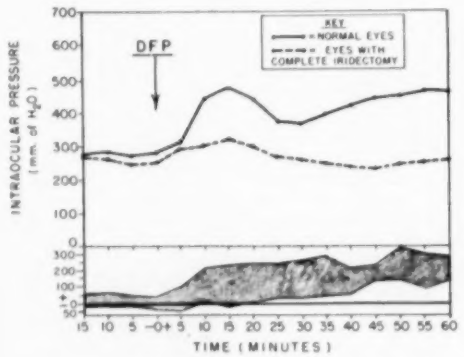


Fig. 13.—Effect of isofluorophate on the intraocular pressure of eyes not operated on and eyes with complete iridectomy.

Lower graph: (1) Shaded area represents confidence region of the difference between medians; significance level 1%. (2) When this shaded area is entirely above the 0 line, the difference between medians is significant.

The compensatory system consists of a pressure bulb operated by a knob and a three-way valve that permits communication of the three essential parts of the instrument with one another.

While the pressure was being recorded, constant and careful observation of the animal and the machine was kept, to account for the variations in pressure produced by the various biological activities (e.g., respiration) not suppressed by narcosis.

The results of the experiments were analyzed statistically, using a significance level of 1%. In two groups, untreated eyes and those that received nitrogen mustard, the ratio t was used to study the significance of the difference between means (Figs. 8, 9, 10, and 11). In the group receiving isofluorophate a nonparametric test for the difference in location between medians was employed (Figs. 12 and 13). This second method was applied to the last group because of the diversity of the readings that made the t test inapplicable.

Results

In the untreated group, the normal eyes had average readings varying between 268 and 247 mm. of H_2O (20 to 18 mm. of Hg). This averaged slightly higher than the pressure curves obtained from eyes operated on, but the difference was not statistically significant (Fig. 8 and 9). Von Hippel and Grünhagen [24] observed the intraocular pressure after complete iridectomy and noted a postoperative fall in the few animals studied.

Nitrogen mustard in the control group produced a maximal elevation to 668 mm. of H_2O (49 mm. of Hg), which was reached approximately 25 minutes after the conjunctival instillation of the drug. The hypertensive process lasted about one to one and a half hours. Figures 10 and 11 illustrate that the maximal readings in the eyes with peripheral iridectomy were around 100 mm. H_2O (7 mm. Hg), and with complete iridectomy about 160 mm. H_2O (12 mm. Hg), less than those in the control group, and the three curves were similar in their general configuration. This difference was significant for the eyes with complete iridectomy, but not for the eyes with the peripheral type of operation.

With isofluorophate the eyes not operated on reached an upper level of pressure values 10 minutes following administration. This level was maintained at 400 to 500 mm. of H_2O (29 to 37 mm. Hg) and was normal when measured after 24 hours. In eyes subjected to surgery, isofluorophate produced only a moderate elevation of pressure, with rapid return to lower levels (Figs. 12 and 13). The values obtained were significantly different from those in the control group.

As can be seen, the hypertension produced by isofluorophate is less precipitous, but more sustained, than the one due to nitrogen mustard. The surgical procedures did not reduce the clinical and histological signs of congestion associated with the administration of the drugs.

It is also interesting to note that with both substances the peak in the pressure readings coincided with the development of maximal miosis. This had been noted previously by Davson and associates ¶ and by von Sallmann and Dillon.[8]

Comment

In these experiments, seclusion of the pupil could not be produced. Visible angle closure did not occur with any of the drugs employed. It is apparent that seclusion of the pupil and angle closure did not occur during the hypertensive phase of nitrogen mustard or isofluorophate actions. Iridectomy diminished the hypertensive reaction to the drugs. Thus, the antiglaucomatous effect of the iridectomy in these experiments is not due to correction of iris bombé, for it did not exist in these experiments. Since iridectomy in rabbits has an antiglaucomatous effect unrelated to physiological seclusion of the pupil and iris bombé, it is possible that the antiglaucomatous effect of iridectomy in humans may not depend exclusively on the relief of iris bombé.

Summary and Conclusion

Physiological seclusion of the pupil did not occur in rabbit eyes.

Seclusion of rabbit pupil with angle closure could not be induced during the hyper-

¶ References 6 and 7.

tensive phase after topical use of nitrogen mustard or isofluorophate (DFP) instillations.

Iridectomy diminished the hypertensive reaction to these drugs.

Iridectomy appears, therefore, to possess some mechanism in addition to relief of iris bombé for lowering elevated intraocular pressure.

Dr. Harry Feilchenfeld assisted in the preparation of this manuscript.

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Stereopsis and Vertical Disparity

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Stereoscopic perception of depth between any two objects in space can be attributed only to the stimuli of the transverse (horizontal) disparity between the images of those objects in the two eyes. Experiment has shown,* however, that for a given retinal region of the two eyes the disparities that give rise to the stereoscopic experience are limited to a definite range; for disparities outside that range there is no patent stereopsis. In the same way that we account for the experimental fact of Panum's areas of fusion, we can also explain the range of disparities for stereopsis by the extent of overlapping of particular arborizations of neurons in the terminal regions of the cortex which arise from disparate retinal elements of the two eyes. This explanation would fit the concept that stereopsis rests on a neuroanatomic and physiologic basis.

We would expect these arborizations to overlap not only in a direction corresponding to the horizontal meridians of the retinas but also in a direction at right angles to these meridians; that is, there would be a two-directional overlap. Consequently, the possible part played by vertical disparities between the images in the two eyes must also be investigated.

There are reasons for believing that stereoscopic depth would not occur when relatively small vertical disparities are introduced between the images of similar objects in the field of view. In the first place, Panum's fusional areas are thought to be smaller in the vertical meridian than in the horizontal, for any given part of the binocular field. Near the fovea the vertical dimension would be of the order of 6 to 8 minutes of arc. Thus, when the vertical disparity is such that the images in the two eyes are perceived double vertically, one might not expect stereopsis on the basis of horizontal disparity to exist, or at least one might expect that it would be much less precise. Indeed, Loy [3] had reported that any vertical disparity introduced between the images resulted in complete loss of stereopsis. The results of recently reported experiments [4] with separated half-images indicated that vertical separations of simultaneously seen half-images sharply reduced stereoscopic acuity. Experiments using a falling bead as the test object under the same conditions, however, suggested that larger vertical disparities could exist, still permitting the experience of some kind of binocular depth perception.

In the early phases of the experiments to be described, therefore, it was somewhat of a surprise to find that relatively large vertical disparities between the images could be introduced without appreciably changing the stereoscopic acuity. The experiments of Lothridge,[5] although difficult to interpret because of his particular instrumental arrangement, did suggest that vertical disparities of 19 minutes of arc might be introduced without causing a failure of stereopsis.

Hering [6] assumed that vertical disparity has no influence on binocular depth perception. At the same time, Helmholtz [7] was of the opposite opinion that when a series of vertical threads are set to appear in a fronto-parallel plane they do not actually lie in that plane because of the absence of vertical disparities of details on the threads which would ordinarily exist with objects in normal surroundings. Hillebrand,[8] however, carefully repeated and amplified Helmholtz' experi-

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* References 1 and 2.

ments, from which he concluded that the latter's results were due to empirical perspective factors, and that stereoscopic depth could not result from vertical disparities. This conclusion has been the one generally accepted to this day. Contributing evidence is to be found also in the data obtained from the three-rod test when these rods are inclined in a frontoparallel plane at various angles (Heine,[9] Monjé,[10] Walker,[11] and others). It can be shown from those experiments that stereoscopic acuity is proportional to the cosine of the angle of inclination,† and therefore is due only to the transverse disparities.

Although vertical disparities introduced between the images of a particular point in space may not affect the stereoscopic depth of that point, it must be kept in mind that a general change in vertical disparities of the images of many separated contours in the visual field, as in asymmetric convergence or by afocal meridional magnifying lenses placed before one eye at axis 180 degrees, does cause an apparent reorientation of all objects in the entire binocular visual field about a vertical axis at the point of fixation.[15] In studying this effect, the vertical disparities introduced by the magnifying lens may become so large as to cause doubling of the images of contours above and below the fixation point, and still the effect continues to operate. This is evidence that large vertical disparities do not inhibit the phenomenon. However, in the specific problem to be considered here the induced effect is not involved.

This paper describes the results of a study of the effect of vertical disparities on stereoscopic depth perception, under controlled stimulus conditions. Obviously, only a point light source can be used as the test object if the basic phenomena are to be studied. In these experiments the stereoscopic acuity was determined by the constant-stimulus psychometric method, with the point light test object exposed for only 0.2 second. The change in that acuity was then determined for increasing steps of vertical disparity introduced between the images in the two eyes until finally stereopsis was lost. The effect was studied for different retinal locations and with respect to different transversely disparate images corresponding to a reference line placed in front of, or behind, the plane of the fixation point. The data show that when vertical disparities are introduced, stereoscopic acuity is only gradually reduced; and stereopsis persists even when the vertical disparities are so large that the test object is seen double vertically.

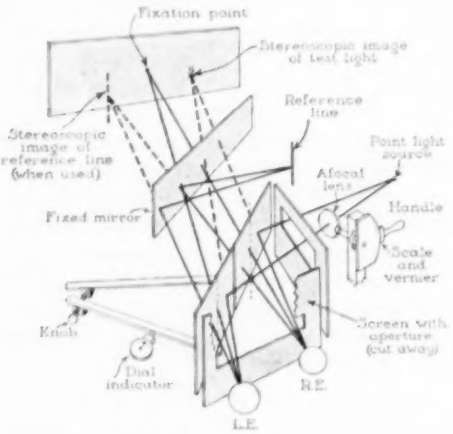

Fig. 1.—Sketch of the apparatus used to study the effect of vertical disparity on stereoscopic depth perception.

INSTRUMENTATION

The basic instrument was the same as that used in other stereoscopic vision studies previously reported.[16] Figure 1 shows schematically the instrument as set up for the present experiments.

The subject's head is held in position by a four-button headrest and a wax bite (a chin cup was used by one subject). The subject fixates a small, brightly illuminated spot on a sheet of plate glass 50 cm. from the eyes in symmetrical convergence, and he also sees the point light source, which in this study is the test object, by reflection from two special mirrors (50% transmission—50% reflection). This point source was obtained from a small hole (0.05 mm.) in a piece of lead foil attached before the window of a suitable small lamp housing. The lamp was so adjusted that the light from the

† The experiments of Weinhold,[12] Kothe,[13] and Depène,[14] in which no depth effect was found for a vertical disparity introduced between the images of the center of three horizontal lines, provide poor contributing evidence, since disparities of this kind at the fixation point would not exist in ordinary surroundings.

pinhole seen by each eye was equally bright. The lamp housing was mounted on an arm (not shown) that could be turned about a vertical axis at a point corresponding to the image of the center of the Vieth-Müller circle. The stereoscopic depth from the images of the point light source could thus be studied at any peripheral visual angle by the appropriate setting of the position of this arm.

When the two mirrors are turned about vertical axes through appropriately small angles in directions opposite each other (by means of levers, a suitable screw arrangement, and a knob), a transverse disparity is introduced between the images of the test point light seen by the two eyes, and accordingly the stereoscopic depth of the test point is changed. The mechanical arrangement that turns the mirrors is such that the image of the test light source seen by each eye is equally but oppositely displaced (the transverse disparity) about a given lateral position on the Vieth-Müller circle. The geometric transverse disparity between the images is zero when the test light is at that position. A dial indicator shows the degree to which the mirrors have been turned, and this is calibrated to read in minutes of arc the angular disparity between the images in the two eyes. An estimated one-fifth of a scale division corresponds to a disparity of 6 seconds of arc.

This instrument design makes possible a wide range of transverse disparities between the images of the test source. Within this range, as the stereoscopic depth changes, the images retain their same brightness, their same angular size and clarity; that is, they do not blur. Empirical clues for change in depth localization of the test images are thus virtually eliminated. This instrument, in contrast to the usual haploscope, uses the same test object, and thus avoids a certain artificiality.

In order, now, to introduce vertical disparities of given amounts between the images of the two eyes from the test point light source, an afocal lens (about 2.5% effective magnification) is interposed in the path of the light from the source to the left eye only. This lens is so mounted that it can be tilted about a horizontal axis (Fig. 1) through any desired angle. Such a lens when so tilted causes a vertical deviation of the image seen through it, without at the same time introducing the chromatic fringe that occurs with the use of prisms. The vertical angular deviation of the image will be of the order of 2.5% of the angle of tilt, and thus disparities of the images of the two eyes up to about 35 minutes of arc were obtained. A calibration of the angular vertical deviation of the image of the point light source seen through the lens corresponding to given angles of tilt of the afocal lens was determined by using a telescope and a vertical millimeter scale placed at the fixation point.

When the effect of vertical disparity on stereoscopic depth for images of the test point was to be studied with a given transverse disparity, the apparent distance of the test point was judged relative to a reference object introduced into the binocular field nearer or farther than the fixation point. This reference object, an illuminated thin vertical needle, was seen by reflection from a large fixed (50% transmission—50% reflection) mirror (Fig. 1). The needle reference object was mounted on a suitable arm that could also be turned about the image of a point corresponding to the center of the Vieth-Müller circle. By suitable rotation of the arm the object could then be placed at any eccentric position; thus, any peripheral retinal location. The reference needle (line) could also be moved along the arm, thereby changing its distance from the eyes.

By means of suitably placed apertures and screens the binocular visual field was restricted to the fixation point, to the images of the reference line (when used), and to the images of the test point light source, all of which were seen against a uniformly illuminated, distant (1.5 meters) white background. The white screen through which the slit aperture was cut was illuminated to the same brightness as that of the background. The experiments were otherwise conducted in a dimly lighted room. Empirical factors leading to spatial localization were thus reduced to a minimum.

The test point light source (and the reference line when used) were exposed simultaneously for only 0.2 second through the entire study. This duration of exposure, which was controlled by an x-ray timer, was short enough to eliminate any marked influence of involuntary eye movements and of fusional movements. It was long enough to be above the threshold for normal stereoscopic acuity. The test light (and reference line when used) were exposed by a suitable push-button contact-switch controlled by the operator.

PROCEDURE

The subject's head was adjusted to the instrument, and the lateral positions of the reflected images of the point light source were adjusted for a given lateral angle and vertical angle relative to the fixation point. The tilt of the afocal lens was set for a desired vertical disparity between the images of the test light point seen by the two eyes.

A constant-stimulus method had to be used to determine the stereoscopic acuity. By trial and error the experimenter determined the range of transverse disparities between the images of the point light sources within which the subject reported (by a hand signal) that the point when exposed appeared "farther" or "nearer" than the fixation point. This range was then divided into

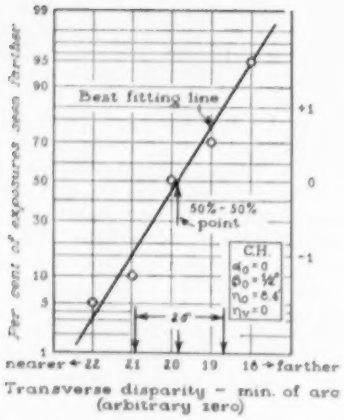

Fig. 2.—Typical set of data plotted on probit coordinate paper. The abscissas represent the transverse disparity introduced, and the ordinates represent the percentage of exposures of a given disparity that resulted in the point test light being seen "farther" than the point of fixation.

five equal steps. Each step, corresponding to a particular transverse disparity, was presented 20 times in a random order. Thus, there was a total number of 100 judgments in each set. In each five successive presentations, however, each of the five steps of transverse disparities of the range would be presented in a random order. With each presentation (0.2 second) of the test point, the subject indicated whether it had appeared nearer or farther than the fixation point. In case of doubt he was forced to make a decision.

The percentage of "farther" judgments in each group was computed from the tally of the number of "farther" and "nearer" judgments made by the observer. A typical set of data is illustrated in Figure 2, where the percentage of the times the exposure of each transverse disparity appeared "farther" (the same could have been found for the "nearer") is plotted on the ordinates of special coordinate paper (probit coordinates) which transforms a normal cumulative distribution curve into a straight line. The best-fitting straight line is determined through the points plotted. By inspection, but preferably by calculation, one can easily find the transverse disparity corresponding to the 50% point (η_{50}). This would represent the most probable transverse disparity between the images of the point light when that point appeared to be the same distance as the fixation point (or reference line).

More important is the determination of the standard deviation of the distribution (σ). This statistic corresponds to one-half the range of transverse disparities, which includes about 68% of the judgments about the 50-50 point. Sigma (σ) is expressed in minutes of arc transverse disparity. This quantity was considered to be proportional to a threshold of stereoscopic perception, and its reciprocal would therefore be a measure of the stereoscopic acuity. Thus, in Figure 2 the 50-50 point, η_{50}, equals 19.8 minutes of arc; the standard deviation, σ, 1.13 minutes of arc. Therefore the stereoscopic acuity, $1/\sigma$, would be 0.885. For the entire series of experiments, η_{50} and σ were obtained by calculation (probit analysis), rather than by graphic inspection, in order to eliminate personal errors of judgment.

This procedure was followed for each of a series of increasing steps of vertical disparity, introduced by the appropriate tilting of the afocal lens, and was continued until stereopsis failed and the judgments became too difficult. In this manner the limiting vertical disparities beyond which no stereopsis existed could be approximately obtained.

Two observers took part in these rather long and tedious experiments. The one (K. N. O.) had considerable experience in making stereoscopic judgments. He wore, in a metal spectacle frame, a +1.25 D. sphere before each eye. The other observer (C. H.) was sufficiently emmetropic (0.50 D. hyperopic) that no spectacles were needed. Her experience in such judgments was limited, and in the present study she was more or less unaware of the nature or purpose of the experiments.

RESULTS

I. *Test Point Light Source Directly Above Fixation Point.*—The effect of vertical disparity on stereoscopic sensitivity was first studied when the test point light source was adjusted to be ½ degree directly above the fixation point. Typical results in which the point light was judged to appear nearer or farther than the fixation point are shown graphically in Figure 3. The quantities η_{50}

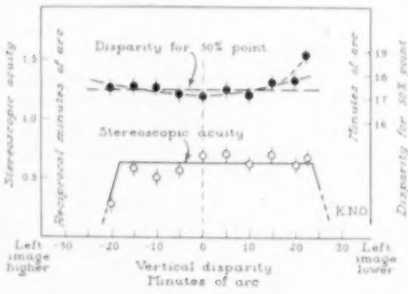

Fig. 3.—Summary of results showing influence of vertical disparities on stereoscopic acuity and depth validity for the test point of light ½ degree above the fixation point. The stereoscopic depth was judged "nearer" or "farther" than the fixation point (K. N. O.).

and $1/\sigma$ are shown on the same graph. The vertical disparities introduced are plotted on the abscissa, to the right of the origin when the image seen by the left eye is lower, and to the left of the origin when it is higher, than the image seen by the right eye. The stereoscopic acuities are plotted on the ordinate axis at the left, and the data indicated by open circles. The disparity corresponding to the 50% level of near and far judgments is plotted on the ordinate axis at the right, and the corresponding data are indicated by filled circles.

The single, outstanding aspect of these data is that a stereoscopic depth perception is maintained in spite of the introduction of relatively large vertical disparities between the images of the test object in the two eyes. In this particular set of data the stereoscopic

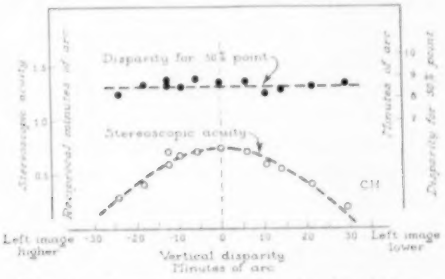

Fig. 4.—Summary of results showing influence of vertical disparities on stereoscopic acuity and depth validity for the test point of light ½ degree above the fixation point. The stereoscopic depth was judged "nearer" or "farther" than the fixation point (C. H.).

acuity is approximately the same throughout the possible range of vertical disparities. The cessation of stereopsis occurred rather abruptly for vertical disparities larger than about 25 minutes of arc. The data show that the validity or consistency of the stereoscopic depth as shown by the 50% disparities is also nearly constant over the range of vertical disparities, though perhaps there may be a slight trend toward increased transverse disparities. The small irregularities in the data must be attributed, at least in part, to the fact that the various sets of data had to be obtained over a period of days, with consequent possible change in the subject's subjective criterion of depth equality.

There was some individual variation in the data obtained by the two subjects, as well as variation for the same subject. For example, Figure 4 shows data of C. H. for the same experimental conditions with the exception that a chin cup was used instead of a wax bite. For this subject the stereoscopic acuity decreased steadily as the vertical disparity increased. It can be shown that this decrease is logarithmic.

One subject (K. N. O.) reported a vertical doubling of the images fairly early in the experiment as the vertical disparities were increased. Since the vertical extent of Panum's areas of fusion when measured is not usually greater than about 10 minutes of arc in the proximity of the fixation point, it is clear that this stereoscopic response persists in spite of the images falling outside the fusional range of disparities, that is, when the images are seen vertically double. The other subject (C. H.) reported doubling only as the limits of the vertical disparity were approached. Both subjects reported that when the test images were seen double, both images participated in the "nearer" or "farther" visual depth experience—the two acting as a single stimulus configuration. It seemed, however, that if too much attention was paid to whether the images were double or not, difficulty in experiencing the stereoscopic depth often resulted.

Subjectively the stereoscopic depth was perceived immediately after exposure of the test point light. Only when the judgments were difficult, for example, near the 50-50 point disparity, or near the limiting vertical disparities, was there any hesitation or feeling for a need of contemplation.

II. *Effect of Transverse Disparity.*—In the course of obtaining data in the experiments described in the preceding section there was a possibility that, as the vertical disparities became large, a response of "nearer" or "farther" could have been learned on the basis of a vernier or nonius stimulus instead of on the basis of stereoscopic depth. If the stereoscopic depth of the test light point is judged relative to a reference object in front

of (or behind) the plane of the fixation point, then this possibility is for the most part avoided. Then the images of the test light point would be transversely disparate.

More important, however, than merely eliminating this factor is the determination of the limiting vertical disparity in the presence of a transverse disparity. If one were to account for the limiting vertical disparities which were found by the extent to which the arborized terminal neurons overlap in the cortex, one might expect a decrease in the limiting vertical disparities in the presence of transverse disparities.

A. The procedure was to set up a refernece object, the small illuminated needle, in front of (or behind) and ½ degree below, the fixation point. Such a reference line would then be seen in the median plane with horizontally (transversely) disparate images. This line and the point light test image above the fixation point were exposed simultaneously by the experimenter. The test light point was then judged by the subject to be nearer or farther than the reference line, while the eyes maintained convergence on the fixation point. Vertical disparities were then introduced routinely as described in the preceding experiment.

With the reference line placed 1 cm. in front of the plane of the fixation point (η_0 = —8.5 minutes of arc), the data for K. N. O. are shown in Figure 5. The coordinates have the same legends as in Figures 3 and 4. In-

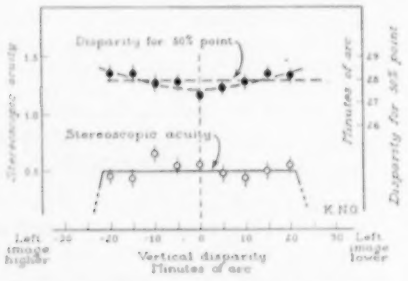

Fig. 5.—Summary of results showing influence of vertical disparities on stereoscopic acuity and the depth validity for the test point of light ½ degree above the fixation point, but when that test was judged "nearer" or "farther" than a reference object 1 cm. in front of the fixation point (K. N. O.).

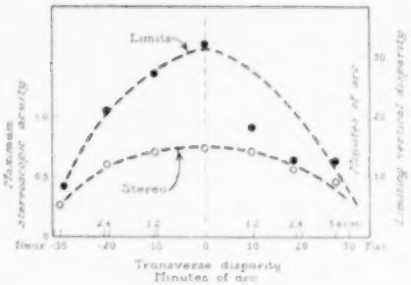

Fig. 6.—Results obtained by C. H. showing limiting vertical disparities and stereoscopic acuities for different transverse disparities between the images in the two eyes, corresponding to certain distances of the reference line placed in front of or behind the plane of the fixation point.

spection of the data shows that while the maximal stereoscopic acuity has been slightly reduced, as might be expected,[16] the limiting vertical disparities beyond which stereopsis is abolished are little changed for these data.

B. When the test point light source was adjusted for a lateral angle but in the visual plane, it was found that transverse disparity did cause some reduction in the limiting vertical disparities, beyond which stereopsis was not experienced. The data for Subject C. H. for a peripheral angle of ½ degree are summarized in Figure 6. The minutes of arc of transverse disparity, corresponding to distances of the reference line behind (to the right) and in front (to the left) of the fixation plane, are plotted on the abscissa. There are two sets of ordinates, one set corresponding to the maximal stereoscopic acuity and the other corresponding to the limiting vertical disparities. Inspection shows that the stereoscopic acuities decrease rather rapidly toward the limits with increase of transverse disparity. For these data the limiting vertical disparities also decrease rapidly with transverse disparity. It must be remembered that these limits are not sharply defined, and yet under the experimental conditions the limiting values given are accurate within 5 minutes of arc. The data were of necessity obtained on separate days, and this alone may result in considerable variation.

III. *Change in the Limiting Vertical Disparities with Peripheral Angle.*—Because the

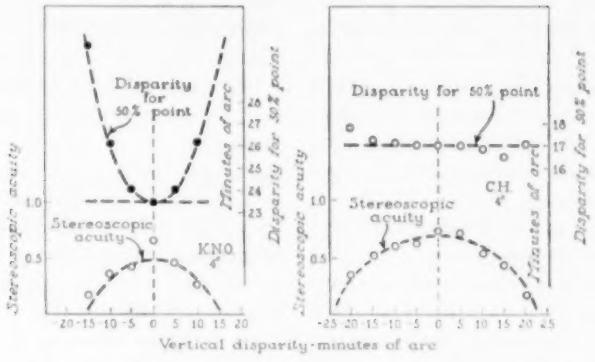

Fig. 7.—Typical results for two subjects showing change in stereoscopic acuity and the validity of the stereoscopic depth caused by introduction of vertical disparities when the test point light was at a peripheral angle of 4 degrees to the right of the fixation point.

magnitude of Panum's areas for fusion, the range of transverse disparities for stereopsis, and the threshold of visual resolution increase (acuity decreases) toward the periphery of the binocular visual field, we might expect the same behavior with the limiting range of vertical disparities within which stereopsis could be experienced. Data were obtained when the test point of light was placed in the meridian of the visual plane at a series of increasing lateral angles. At each angular position, the stereoscopic acuity for the complete range of vertical disparities and the limiting vertical disparities themselves were determined.

The results for the two subjects were unlike in some respects. In both, however, the curve for stereoscopic acuity versus vertical disparity was bell-shaped, and in some instances sharply so. Figure 7 shows two sets of data selected for the 4-degree peripheral angle. The interesting aspect of these curves is that the disparity corresponding to the 50-50 point increased rapidly for K. N. O. but remained constant for C. H. as the vertical disparity increased. In the first case the test light is seen increasingly nearer, necessitating increased crossed transverse disparity. This behavior must be attributed to a decreased stereoscopic response and the influence of some empirical factor associated with the brightness of the test light point on the conception of depth.

Figure 8 summarizes results for one subject. It shows the change in stereoscopic acuity and the change in the limiting vertical disparities for increasing peripheral angles. The peripheral angles at which the test light point is located are plotted on the abscissa, and the stereoscopic acuity is plotted on the ordinate at the left. The limiting vertical disparities are plotted on the ordinate scale at the right.

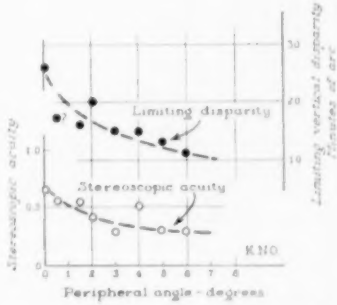

Fig. 8.—Graph for one subject (K. N. O.) showing change in the stereoscopic acuity, but, more importantly, the change in the limiting vertical disparity with peripheral angle.

The fall in stereoscopic acuity for this subject was expected. However, the definite fall in the limiting values for the vertical disparity with peripheral angle was not expected. The results for subject C. H., however, show a less marked decrease in the stereoscopic acuity with the peripheral angles up

to 8 arc degrees, and, moreover, a nearly constant limiting value for vertical disparities was found.

The important aspect of these results is not the variation between the two subjects but, rather, that the limiting vertical disparities do not increase with peripheral angle (and may actually decrease).

Data were also obtained when the point light source was adjusted to a lateral and vertical position in relation to the fixation point. Essentially the same results as those presented above were obtained. Of special interest was the fact that the stereoscopic depth remained valid and consistent, as shown by the 50% disparities in spite of the vertical disparities introduced. Thus there was no evidence of the operation of the induced effect. One might suspect (1) that the 0.2-second exposure is too short for the induced effect to operate, and (2) that the contours involved were too limited to initiate the effect strongly.

COMMENT

The experimental set-up described here provides the fundamental conditions for testing the influence of vertical disparities on stereoscopic depth. A point light source was used as the test object, and as used no empirical clues to spatial localization occurred as its stereoscopic depth changed with transverse disparities. More complicated test objects (for example, that used by Lothridge [5]) obscure the basic aspects of the problem. In spite of the vertical disparity between the images, and even when these images were seen double, the response to changed transverse disparities must indeed have been a true stereoscopic depth response. Certainly the form sense was not involved, nor was retinal resolution directly involved, except in that visual acuity may set a boundary condition.

The unexpected aspect of the results obtained is that relatively large vertical disparities, even near the foveas, do not abolish stereoscopic depth. Even when the vertical disparity is so great that the images are seen double vertically, the two images act as a single configuration and a steroscopic depth is experienced.

The only experimental results that may bear on the influence of vertical disparities were those found in the induced effect.[15] In those experiments with a tilting board with restricted contours at equal distances above and below the fixation point, the induced effect (the apparent rotation and distortion of the entire binocular visual field about a vertical axis through the point of fixation, when the image of one eye is magnified in the vertical meridian only) occurs even when the difference in magnification of the images is so large as to cause the images of the separated contours to appear double. The magnitude of the effect, however, is reduced. In those experiments, for example, vertical disparities of 20 minutes of arc for a vertical visual angle of 5 arc degrees gave rise to the maximal effect.

The present experiments show also little deterioration in the validity of the stereoscopic depth as the vertical disparities are increased.

In the normal use of our eyes in customary surroundings, only objects close at hand, and those above or below the visual plane, can give rise to images that are vertically disparate. The images are vertically disparate because the distance from the point to one eye is farther than that from the point to the other eye, and hence the point subtends unequal angles to the two eyes. The vertical disparity angle (η_v) is the difference in the two vertical visual angles subtended by the distance from the visual plane to the point. The only exception to this rule is when the points lie in the median plane; thus when the eyes are in symmetrical convergence, points directly above or below the fixation point will have images in the two eyes which are not vertically disparate. In asymmetric convergence, however, the images of points above or below the fixation point are vertically disparate. Similarly, lenses which magnify the image of one eye compared with that of the other in the vertical meridian will

likewise introduce vertical disparities between the images of all points in the binocular visual field except those in the visual plane, the disparities increasing the greater the vertical visual angle subtended by the point.

The vertical angular disparity described above is obviously a "geometric" disparity. The effective, or physiologic, vertical disparity between the images of these points may be smaller or be greater than the geometric disparity, depending on other factors, such as the neuroanatomic asymmetries between the two retinas.[17]

However, apart from neuroanatomic asymmetries, an effective vertical disparity may normally be introduced (*a*) by a disjunctive cyclotorsion between the eyes, so that extrafoveal points, even in the visual plane, will be vertically disparate, and (*b*) by a vertical fixation disparity. Then a constant vertical disparity (rarely greater than 6 to 8 minutes of arc) is added to all those vertical disparities which would already exist for all images of points in the binocular visual field.

Thus it should be clear that vertical disparities between the images of the two eyes from objects close to the fixation point never occur in the normal use of the eyes, or are so small that they are below visual resolution. Yet according to the data obtained in these experiments, stereoscopic depth is experienced when large vertical disparities are introduced between the images of objects near the fixation point. This is an important fact in our attempt to understand stereoscopic depth perception and its origin. The stereoscopic depth perception of points near the fixation point, when a vertical disparity is introduced, would not result from learning to interpret stimuli in the particular structures involved. Yet we must agree that neuroanatomic structures must be present for such a stereoscopic response, though such structures had never before been utilized in this unusual manner. This conclusion would suggest an innate character of the origin of stereoscopic vision. The alternative explanation would be that stereoscopic depth is the result of a generalized integrative process, based on experience, and that the experience of depth readily follows a certain type of stimulus—in this case, a transverse disparity, even though the images may also be vertically disparate. The existence of fairly definite limits in the extent to which both transverse and vertical disparities may be introduced without abolishing stereoscopic depth certainly indicates the existence of limiting structures, and as such implies a neuroanatomic limitation and a neurophysiologic process for stereopsis.

Based on the hypothesis that the range of disparities within which stereoscopic depth can be perceived lies in the reciprocal overlap of the arborization of terminal neurons in the cortex associated with disparate retinal elements in the two eyes, our data would imply the following conclusions:

1. The magnitude of the transverse meridians of the areas of overlapping for stereoscopic perception is greater than that for fusion (Panum's areas). Moreover, the magnitude of the transverse dimensions of the stereoscopic areas increases more rapidly for images falling on the peripheral parts of the retinas than does the magnitude of those for fusional areas.

2. The magnitude of the dimensions of the overlapping for stereoscopic vision corresponding to vertical meridians is much larger than that for fusion. However, the vertical dimensions of the stereoscopic areas are greatest near the fixation point, and may decrease for images falling on the peripheral parts of the retinas in the meridian of the visual plane.

While the comparative results essentially describe the relative dimensions of hypothetical neuroanatomic counterparts concerned with fusion and stereoscopic depth perception, and do suggest that stereopsis may not be associated with fusion per se,[18] they do not constitute proof. Roelofs[19] suggested that most central impulses for eye movements, which would result from the stimuli of transverse disparity, should be considered as the physiologic correlative of optical localization. Whereas Roelofs had in

mind the innervations for fixation movements, it is more probable that the disparity would lead to innervations for fusional movements. The fact that the half-images may be seen double and stereopsis still persists does not preclude the stimulus for fusional eye movements, movements which cannot take place because of attention to the point of fixation.

SUMMARY

An experimental arrangement is described which permits the determination of stereoscopic depth acuity and validity, using a point light source as test object, when vertical disparities are introduced between the images of the light source seen by the two eyes. The results show that despite unexpectedly large vertical disparities (up to 25 minutes of arc) stereopsis still exists, and that the stereoscopic depth will be valid. This range exists even when the test point is near the fixation point. In general the limiting vertical disparities beyond which stereopsis does not occur decrease with the transverse disparity and, with some individual variation, decrease as the test light is placed farther and farther from the fixation point in peripheral vision. These results are discussed with special reference to the overlapping of neurons in the cortex and to the origin of stereopsis.

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Treatment of Retinoblastoma by Radiation and Triethylenemelamine

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We have been treating retinoblastoma by radiation according to a method described by us first in 1936.[1] The technique, which was devised by Dr. Hayes E. Martin, Memorial Center for Cancer and Allied Diseases, includes the employment of special cones at a temporal and a nasal portal in an effort to deliver an adequate dose to the posterior sector of the eye, to the exclusion of the vulnerable anterior sector. Subsequent reports on the use of this technique were made in 1942,[2] 1945,[3] and 1949.[4] The treatment is employed for patients with bilateral tumors whose eye with the more advanced disease has been enucleated. The less involved eye has been treated when it was thought that useful vision might be salvaged if the tumor could be arrested. Since the institution of the treatment in 1936,[1] a total of 148 patients have been treated. Forty-three patients have died of the disease, and 26 patients have been lost to follow-up (Table 1). The results of the treatment of the surviving and documented 79 patients (80 eyes) are shown in Table 2. It can be seen that useful or limited vision as defined by us was obtained in 36.2% of the eyes. With this treatment cataract has not been a problem; so for the most part the irradiation apparently does avoid the lens.

The complication which has given us greatest concern from the beginning has been late vitreous hemorrhage from retinal blood vessels presumably damaged by irradiation. These late hemorrhages have appeared many months to a year and a half after completion of the treatment and in many instances have marred what seemed to be satisfactory results with useful vision. For a number of years we tried to avoid this discouraging complication by reducing the amount of radiation to the lowest point which would be lethal for the

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TABLE 1.—*Treatment of Bilateral Retinoblastoma by Radiation*

	No.
Surviving	79
Died of the disease	43
Doing well at last examination but lost to follow-up	19
Not doing well at last examination but lost to follow-up	7
Total	148

TABLE 2.—*Results of Treatment of Retinoblastoma by Radiation*

No. of Eyes	Years Follow-Up	Vision
13	1-3	Useful
2		Limited
6		None
2	3-5	Useful
0		Limited
14		None
0	5-10	Useful
4		Limited
24		None
4	10+	Useful
4		Limited
7		None

Total 80 eyes (79 patients)

Useful vision	19 (23.7%)	(36.2%)
Limited vision	10 (12.5%)	
None	51 (63.8%)	

tumor. The dose was gradually reduced from 8000 r/air to each of two ports, the amount originally advised, to 4400 r/air to each, which seemed to be adequate to arrest the growth, but late vitreous hemorrhages continued to occur occasionally. We were questioning whether or not our x-ray dose could be reduced even lower when, through the courtesy of Dr. Alan Woods, we saw a patient with advanced disease that had shown a remarkable regression with the use of radiation in combination with nitrogen mustard therapy. This was reported by Dr. Carl Kupfer.[5] Combining x-ray treatment with a radiomimetic drug seemed to offer a good opportunity to reduce considerably our x-ray dose. After consultation with the Radiotherapy Department of the Presbyterian Hospital, we decided to use triethylenemelamine (TEM) because its oral administration, in contrast to the intravenous administration of nitrogen mustard, is a distinct advantage for small children. Furthermore, the experimental work of Gillette and Bodenstein[6] added support to our premise. They exposed amphibian embryos to a close ally of TEM, namely methyl-bis (β-chloroethyl) amine (MBA), and found that the drug has a specific effect on the proliferating retinal cells. In Figure 1, *b* and *b'* show the selective action on the growing retinal cells 1 and 3 days after exposure, and *d* and *d'*, 5 and 15 days after exposure. It can be seen that the drug has a great specificity for the retinal cells, largely to the exclusion of the other tissues. The retinal elements show a striking progressive degeneration and necrosis from the 1st to the 15th day. The authors concluded that the drug had no effect upon cells already on their way to differentiation, while proliferating cells had their mitotic activity abolished and then degenerated. This would indicate that the nitrogen mustard group would be more effective in the usual retinoblastoma composed of undifferentiated retinoblasts than in the rarer neuroepitheliomatous type of retinoblastoma (rosette formation), composed mostly of more differentiated cells. This is borne out in one of our patients (Fig. 4).

Therefore, in January, 1953, we started to treat all suitable patients with retinoblastoma by the combination of radiation and TEM. Most patients treated were instances in which the eye with the more advanced tumor was enucleated and the eye with the less advanced lesion was treated. The criterion always was whether or not useful vision could be retained if the tumor were arrested. If not, then, of course, enucleation is preferable. We have in our series four patients in whom both eyes have been treated. These tumors were detected sufficiently early for this treatment, rather than enucleation, for the following reasons: 1. The mother was a survival from retinoblastoma. She had one sibling with bilateral disease; so the patient we treated was examined routinely in infancy and relatively early lesions were found in each eye. 2. The mother was a survival from retinoblastoma, and the pediatrician therefore examined the child's eyes for tumor and found relatively early lesions in both eyes. 3. The mother detected in her child a white reflex in one eye, where the tumor was located at the posterior pole around the optic nerve. 4. The patient was examined because a sibling had retinoblastoma, and relatively early lesions were found in each eye.

In the past 21 months we have treated 34 eyes with radiation and TEM according to the method described here. Nineteen of these eyes have shown satisfactory regression (Fig. 2). In addition, we have treated eight eyes in which the lesions showed a good response in the beginning but after a period of time, ranging from six to eight months following the completion of the treatment, the tumors manifested active growth (Fig. 3). Additional therapy to these eight eyes was instituted, and in three instances the lesion has regressed satisfactorily.

There were some extenuating circumstances connected with the therapy of four of these eight eyes mentioned above. 1. Two eyes (a bilateral case) were treated with TEM for six weeks before x-ray therapy was started. The radiation to each eye was given through only one port—the temporal one. 2. One eye was treated through only

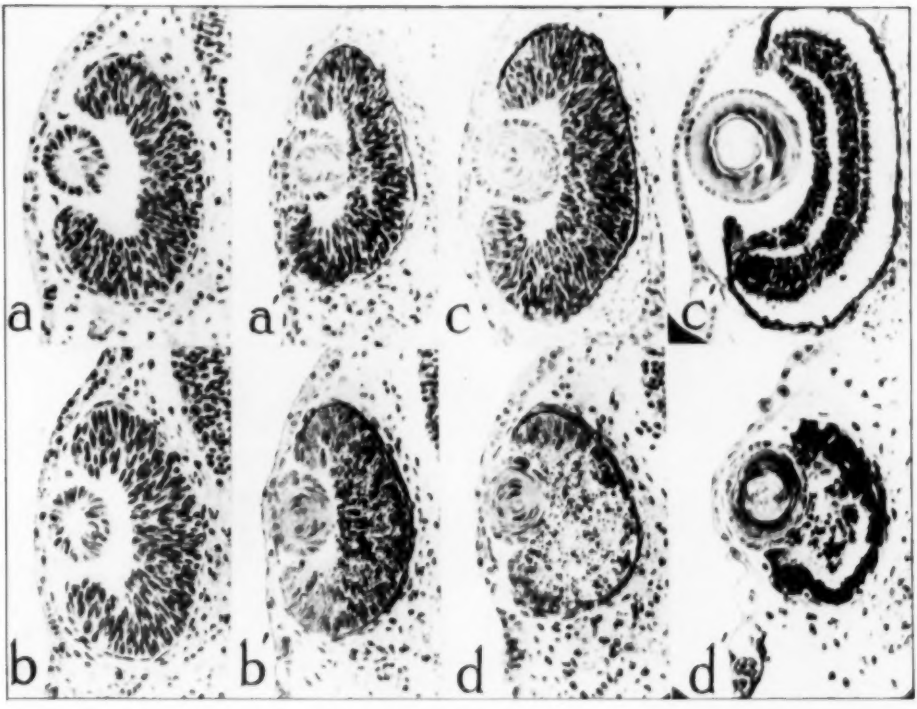


Fig. 1.—Effect of nitrogen mustard compound (methyl bis[β-chloroethyl] amine; MBA) on the growing retinal cells of a salamander embryo.

a and *a'* are the control eyes one and three days after exposure.

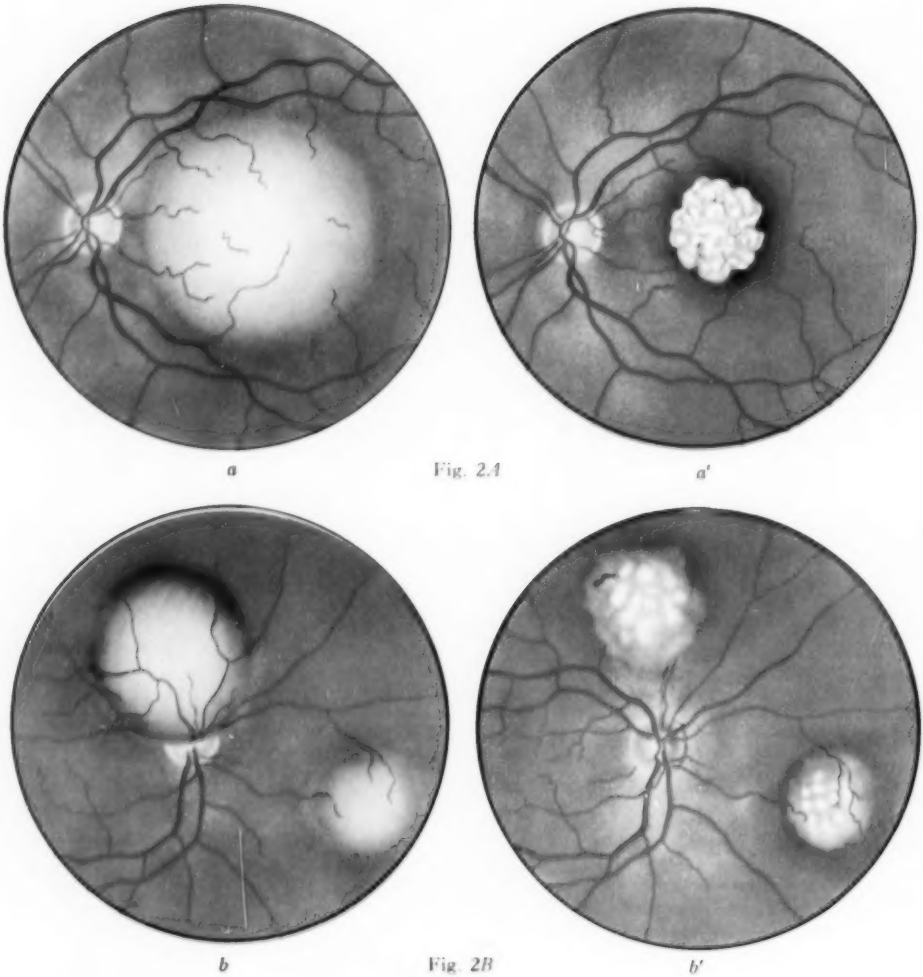
b and *b'* are the exposed eyes one and three days after exposure.

c and *c'* are the control eyes 5 and 15 days after exposure.

d and *d'* are the exposed eyes 5 and 15 days after exposure.

The retinal elements show a striking progressive degeneration and necrosis from the first to the fifteenth day.

Reproduced from Gillette, R., and Bodenstein, D.: J. Exper. Zool. **103**:1-32, 1946, published by the Wistar Institute of Anatomy and Biology, Philadelphia.



a Fig. 2A *a'*

b Fig. 2B *b'*

Right Eye

Fig. 2.—Appearance of some successfully treated eyes to date before and after treatment.

Aa, the lesions before treatment.

a', the lesion one year eight months after treatment.

B, a bilateral case.

Right eye:

b, two lesions in the right fundus before treatment.

b', the same lesions one year seven months after treatment.

Left eye:

c, the lesion in the left eye before treatment.

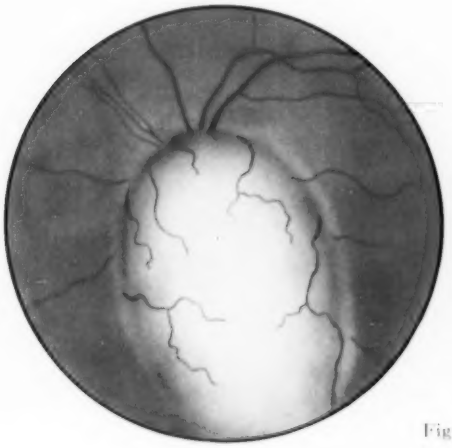
c', the same lesion one year seven months after treatment.

Cd, three lesions before treatment.

d', the same lesions six months after treatment.

De, one large lesion, a diffuse involvement in the periphery above, and several daughter implantations on the retinal surface.

e', the same lesion five months after treatment.

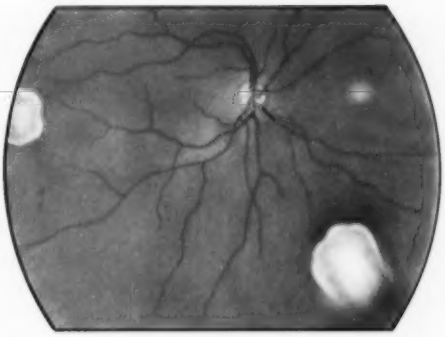


c

c'

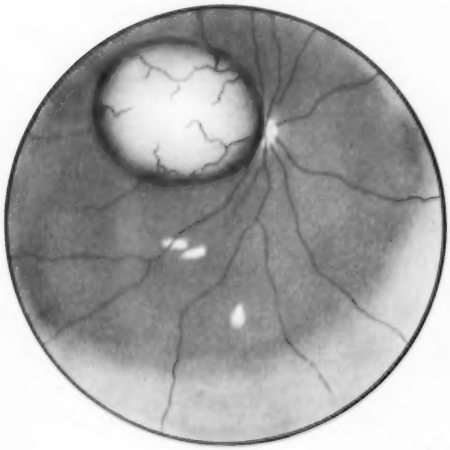
Fig. 2B
Left Eye

d

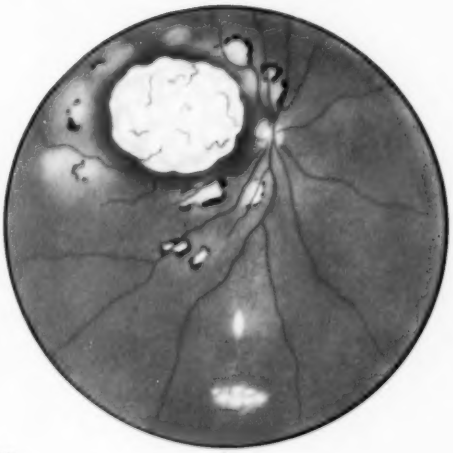


d'

Fig. 2C

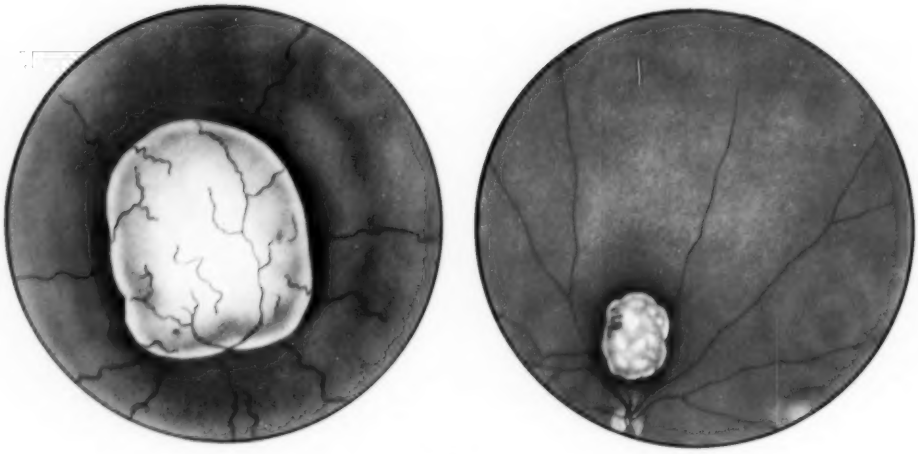


e

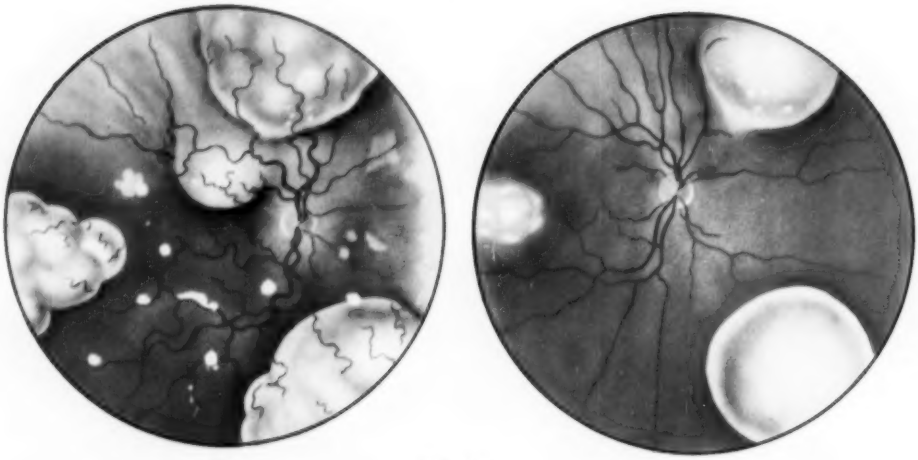


e'

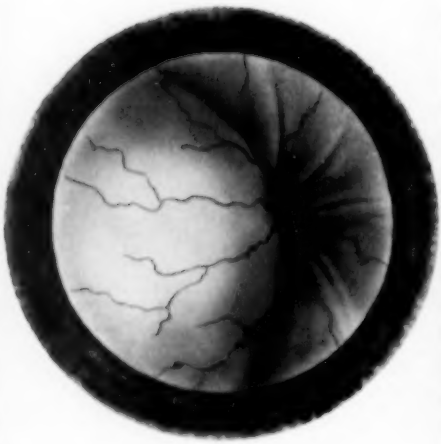
Fig. 2D



a Fig. 3*A* *a'*



b Fig. 3*B* *b'*



a

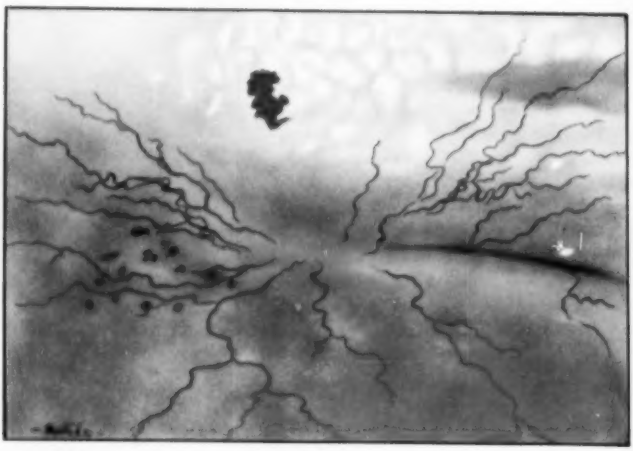


Fig. 4 *b*

Fig. 3.—Appearance of the eyes in two cases which have shown active growth after the completion of the first course of treatment. These have been given further treatment, and to date the regression has been satisfactory.

Aa, one large lesion which covers the disc before treatment. There was what seemed to be a flat detachment of the retina.

a', the same lesion six months after completion of treatment. The retina appears reattached.

Bb, multiple lesions before treatment.

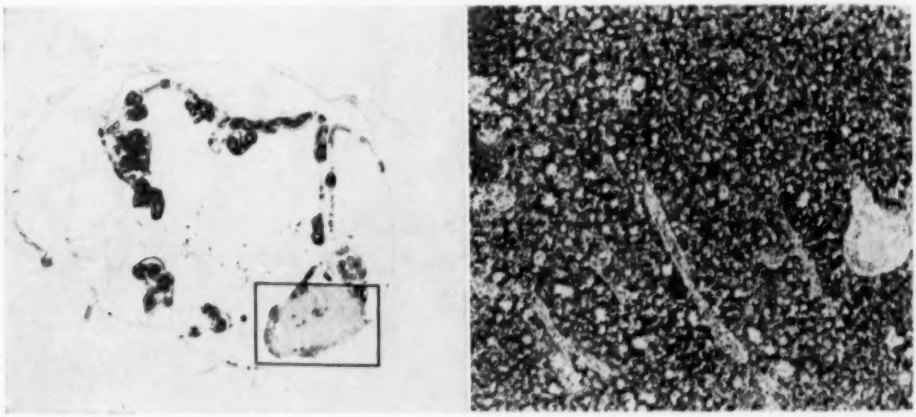
b', the same four months after treatment.

Fig. 4.—An eye in which the differentiated area of the tumor (neuroepitheliomatous portion with rosette formation) was relatively unaffected by therapy. In this patient an enucleation of the eye with the more advanced lesion was done.

The parents were advised that the other eye should also be enucleated because of advanced disease, but they elected to let the tumor take its course. After two weeks they noted a marked reduction in vision of the remaining eye, due to tumor growth. There was a complete detachment of the retina with extensive tumor involvement. As the parents desired any treatment short of enucleation, TEM was given from Jan. 29 to March 30, 1953, and during this period there was marked regression. This was estimated at about 80%. X-ray treatments were then started on March 30 and completed April 27, 1953, and the TEM was continued. The regression was so marked that on Oct. 5, 1953, we have a note to the effect that the patient could throw and recover a ball, could play with toys, and picked up food from the table and tray, as well as leaves from the lawn. On Nov. 23, 1953, we noted an increase in the size of the residual tumor and more radiation was given. On Feb. 15, 1954, because any useful vision seemed to be precluded, the parents consented to enucleation, and this was carried out.

(*a*) Appearance before treatment was started. There is a complete detachment of the retina. The portion above appears solid.

(*b*) Appearance on Dec. 28, 1953.



c *d*

Fig. 4.—(*c*) The enucleated eye. The area encircled was relatively unaffected by the treatment, while elsewhere the tumor appears necrotic.

(*d*) High-power view of the encircled area to show differentiation in the form of rosettes.

one port—the temporal one—and this was directed too much posteriorly. 3. One eye received curtailed treatment because the patient was exposed to poliomyelitis.

Therefore, out of the 34, there are 22 eyes * in which we feel that the tumors are arrested and that the eyes have good vision.

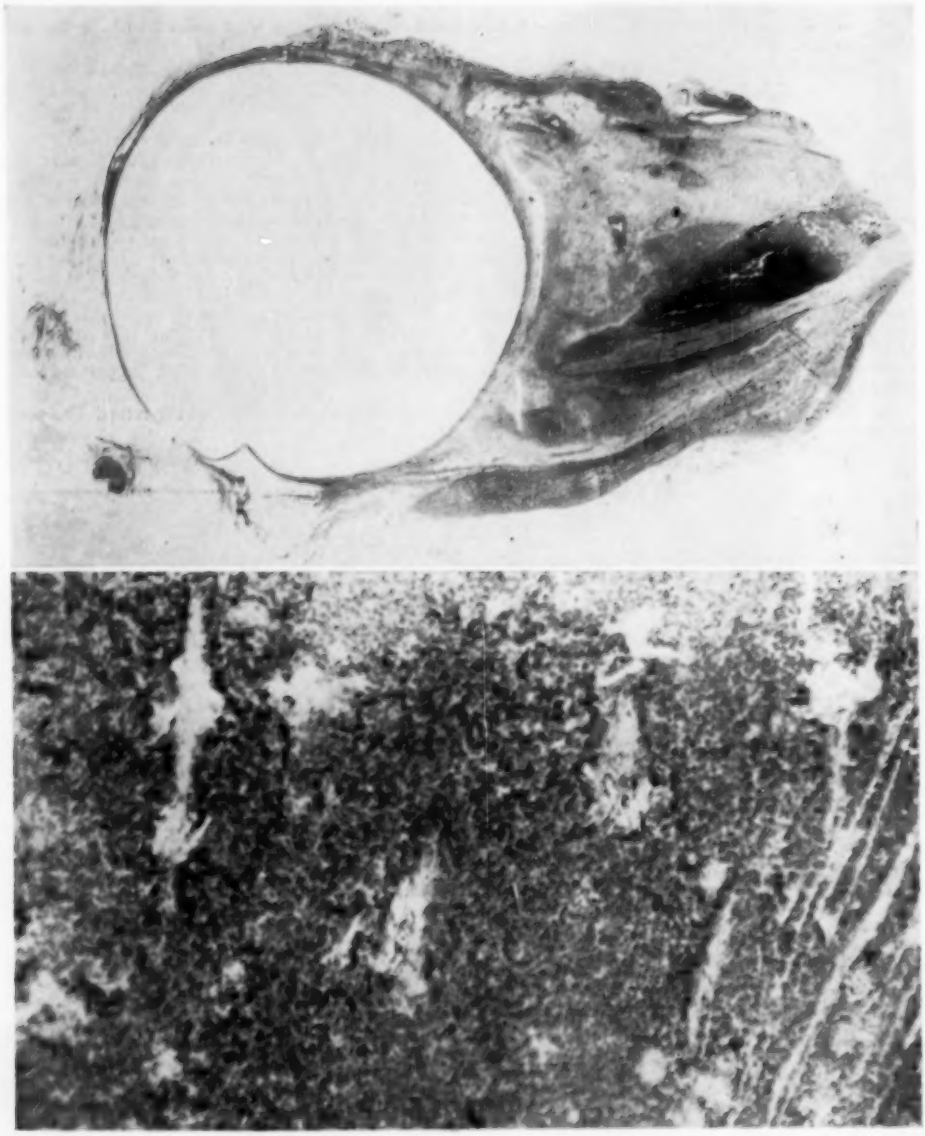
The status of the remaining 12 eyes of the total 34 is as follows:

(*a*) Three eyes † had additional treatment, and the lesion has shown no further regression.

* This report was made as of Sept. 15, 1954. During the six additional months of follow-up (as of March 15, 1955) with regular examinations under chloroform anesthesia, there has been no growth of the tumor in any of the 22 eyes.

† In these eyes the tumor was not controlled, and enucleation has either been done or advised. A feature in common to these eyes was tumor seeds floating in the vitreous. This seems to occur only when the tumors are large and extensive. It seems probable that the effect of the TEM does not reach these seeds in the avascular and inert vitreous.

a



a'

Fig. 5.—Sections of recurrent tumor in the orbit and a lesion in the fellow eye eight months after treatment by radiation and TEM.

(*a*) Necrotic tumor in the muscle funnel of the left orbit behind the round, empty fibrous capsule which surrounded the implant.

(*a'*) High-power magnification of the above. The tumor cells have lost their staining qualities and do not appear viable.

(*b*) Two eyes† have not had the additional treatment completed.

(*c*) Three eyes were considered unsatisfactory for treatment.

1. One had radiation (6000 r/air to each of two ports) elsewhere, and when we saw the patient there was a cataract which prevented a view of the fundus. We had no idea, therefore, of the status of the growth. We gave the patient TEM and no more x-ray treatments. Intractable glaucoma developed, and the eye had to be enucleated.
2. One eye received x-ray treatment (5200 r/tumor dose) elsewhere, and when we examined the patient the vitreous was cloudy with hemorrhage, so that we could not get a view of the fundus. We gave TEM alone in the hope that when the hemorrhage absorbed we would find an arrested tumor. When the vitreous cleared sufficiently to permit us to see the fundus, we found the entire retina involved with tumor, which also filled part of the vitreous cavity. As no vision would be possible, even though the tumor were arrested, we advised enucleation.
3. One eye showed an extensive growth, with complete detachment of the retina, and enucleation was advised, but as the parents refused it (Fig. 4), treatment was given.

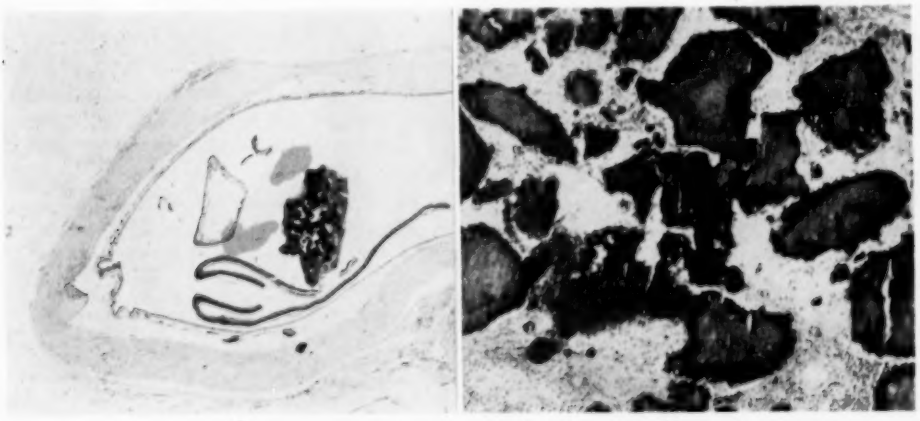
(*d*) Two eyes were treated and although the lesions responded most satisfactorily, the patients died of metastasis.

1. The eye with an advanced tumor was enucleated. The fellow eye was treated by radiation and TEM, and the tumor regressed. The patient died of metastasis after only two weeks of treatment. At autopsy, tumor extension from the anophthalmic orbit was found in the subdural space and brain.
2. The eye that was treated showed the lesion in what we considered an arrested state for eight months before the patient died of extension of the tumor from anophthalmic orbit (Fig. 5*b*, *b'*).

(*e*) Two eyes in which the tumor is out of control and the prognosis is poor.

The accepted amount of time has not elapsed since the completion of treatment to justify the claim of cures in the *22* eyes cited above. There have been two to two plus years since the completion of the x-ray treatment in nine eyes; one and one-half to two years since completion of treatment in four eyes; one to one and one-half years in five eyes, and one year and under in four eyes.

From our experience with the treatment of retinoblastoma with radiation alone, we would say that the lesion in these eyes at the present time have the appearance that we have been accustomed to associate with arrested disease (Fig. *2*). Furthermore, we



b *b'*

(*b*) The lesion in the right eye. The patient died of intracranial extension from the recurrent tumor in the left orbit eight months after completion of treatment. The section is through the lesion in a collapsed globe obtained at autopsy. The tumor is reduced to almost solid calcium.
(*b'*) High-power view of section through the lesion to show the calcium and necrotic tumor.

can say that if a retinoblastoma is not under control by treatment it becomes manifest usually in three to six months. We think this is true for two reasons. The one is that we are viewing isolated lesions in transparent media, and the other is that we are dealing with relatively small lesions which can be scrutinized carefully when magnified × 16 with the ophthalmoscope. Therefore, active growth of the tumor can be appreciated in a relatively short period

In only one patient have cataractous changes been noted to date. This patient had

This is why in approximately one-third or more of the cases the tumor is bilateral. It is also frequent to see multiple origins in the retina of the same eye, and in the series reported here 50% of the eyes had multicentric foci of tumor. We believe that this feature militates against successful treatment by radon plaques. Also, vitreous tumor seeds, a feature seen in some eyes, would probably not be reached through radon plaques.

We have treated eight additional, but different, types of cases (Table 3) in the

TABLE 3.—*Summary of Eight Cases with Tumor Invasion of the Optic Nerve*

Name	Type of Case	Post-operative Irradiation to Orbit	Irradiation to Fellow Eye	TEM Therapy	Orbital Recurrence	Comment	Results
D. E.	Unilateral	Yes	No	Oral (complete)	Yes	.....	Intracranial and general metastasis Died
P. Mc.	Unilateral	Yes	No	Oral (incomplete) Intra-arterial to orbit	Yes	.....	Intracranial and general metastasis Died
W. D.	Bilateral	No	Yes	Oral (incomplete)	No	Died 6 wk. from first visit	Intracranial and metastasis to meninges Died
K. B.	Bilateral	No	Yes	Oral (incomplete) Intra-arterial to orbit	Yes	.....	Intracranial metastasis Died
D. W.	Bilateral	No	No	Intra-arterial to fellow eye	No	TEM preceded planned enucleation by 7 days	Living No symptoms
A. M.	Bilateral	No	Yes	Oral (under treatment)	No	Tumor in distal portion of nerve; massive tumor in fellow eye	Living Prognosis poor
R. H.	Bilateral	Yes	Yes	Oral (under treatment)	No	Massive tumor in fellow eye	Living Prognosis poor
N. A.	Bilateral	No	Yes	Oral (complete)	No	Tumor in distal portion of nerve	Living and well

treatment completed over a year and one-half ago. At the present time there are slight posterior cortical changes. No retinopathy has been noted, and no vitreous hemorrhages have occurred.

We do not have visual acuity on treated patients because they are practically all too young for an accurate visual determination. We would expect good vision in all these patients should the neoplasm prove ultimately to be arrested.

It is well known that an outstanding characteristic of retinoblastoma is multiple origin.

past 21 months with radiation and TEM, and they are as follows: Six patients in whom residual tumor was left in that portion of the optic nerve remaining in the orbit after enucleation and two patients in whom the tumor was found in the distal portion of the excised optic nerve but probably was not in the proximal portion.

(*a*) Two of these eight patients have died of intracranial extension, and the fellow eye remained normal throughout.

(*b*) Two patients died of intracranial extension, and the fellow eye had been treated with x-rays and TEM, with satisfactory

regression. (These are the two cases mentioned in *d* (page 509).

(*c*) Two patients have advanced tumor in the remaining eye treated with radiation and TEM, and, although both are alive, with no trouble imminent, the prognosis is poor.

(*d*) One patient is living and well, with no apparent trouble.

(*e*) One patient had a very advanced tumor in each eye. The right eye was enucleated, and we recommended enucleation of the left eye but before the parents could give us their decision 11 days elapsed, during which we used TEM to observe what effect it would have on the growth. This eye was enucleated.

Patients for whom previous types of therapy have proved completely inadequate and who have generally been viewed as hopeless are now being treated by intra-arterial TEM in combination with radiation. In this category fall the cases with residual or recurrent tumor in the orbit or cases in which the intraocular tumor has proved refractory to the usual course of treatment. It is too early to make any statement regarding the effectiveness of our treatment of these patients.

METHOD OF TREATMENT

The therapy program has two distinct aspects, each of which will be discussed.

1. *Radiation.*—The factors employed are as follows: 220 kvp; 0.5 mm. copper plus 1.0 mm. aluminum filtration (H. V. L. 1.0 mm. copper); 20 ma.; 50 cm. T.-S. D.; a 2.5 cm. temporal port and a 2.0 cm. nasal port; 400 r/air per treatment given three times a week to alternate portals for a total of 2400 r/air to each within one month.

The exact method of administration and the means by which the patient is immobilized during therapy have been fully described in previous publications.‡

The tumor dose is calculated to be approximately 3400 r, in contrast to a tumor dose of 6200 r which was administered formerly when radiation alone was employed. The new figure is such that there is reasonable doubt that it alone would be as successful in arresting the disease as the previous method had been. Therefore, if the survival rate were maintained or bettered, it would be reasonable to attribute the efficacy of the new therapeutic approach to the addition of the triethylenemelamine. Whether the dose of x-rays can be further reduced will be determined when the final effects of the present method have been fully evaluated.

2. *Triethylenemelamine.*—Triethylenemelamine (TEM) is an analogue of nitrogen mustard, a drug developed first in 1946 by Gilman and Philips.[7] These agents have proved useful in the management of some diseases of the hematopoietic system, Hodgkin's disease, and allied disorders, when these conditions become generalized or radioresistant. TEM has been used almost exclusively by our group because, unlike nitrogen mustard, it can be given orally, a necessity in infants and young children, who compose the group affected by retinoblastoma.

Since it was found by experience that TEM is tolerated surprisingly well by children, an average oral dosage schedule was set up somewhat as follows: In children under 12 months 2.5 mg. is given; from 12 to 18 months, or under 20 lb. (9072 gm.) in weight, an initial dose of 3.0 mg. is given if a preliminary survey, including complete blood count and platelet count, is satisfactory. A dose of 3.5 mg. is administered initially to older or larger children. Complete blood and platelet counts are repeated every two weeks, with maximum depression usually noted at four weeks. The second and subsequent doses of treatment are given after a suitable interval, when the white cell count has returned to a level of at least 5,000, and preferably 6,000, and the platelet count to 150,000, a point usually reached six to eight weeks after the last treatment. The subsequent doses selected depend upon the depth of hematologic depression and the rate of fall.

The total course administered is approximately 15.0 mg., given in five divided doses.

‡ References 1 through 4.

The first dose is given at the start, and the second at the conclusion, of x-ray therapy. In one instance TEM was given six months after completion of x-ray therapy, with a remarkable improvement, suggestive of synergism. It was given alone initially in several advanced cases; and when unexpected improvement occurred, x-ray therapy was added to the regimen. For the most part, treatment has been concurrent. As yet the optimal timing of radiotherapy and TEM effect has not been established.

The drug, supplied by Lederle Laboratories, when administered orally, is given on an empty stomach, usually at 8 a.m., after an eight-hour fast, with 0.6 gm. of sodium bicarbonate (Gellhorn and associates [8]). Nothing is given orally for three hours thereafter to insure a maximum, even absorption. Immediate toxic effects noted included occasional vomiting in 10% of the children and fever, with a temperature of from 100 to 102 F, within 24 hours of treatment in 10%, usually in the youngest children. Late changes were those of the usual hematologic depression described by Rhoads, Karnofsky, and associates,§ with leucopenia and lymphopenia followed by thrombopenia and anemia, in that order. A moderate eosinophilia occurred occasionally.

As in any therapeutic program, stress must be placed on individual variation. Some children are extremely sensitive, and it is only by the utmost vigilance that dangerous or fatal hematologic depression can be prevented. This was avoided in more than 150 separate treatments in all of the 30 children treated orally.

As our experience with the agent increased, we have recently added the intra-arterial route of treatment to our armamentarium in advanced or previously refractory cases. The level of the chemotherapeutic agent reaching a localized tumor via the regional arterial supply can be many times greater than that given orally (Klopp [11] and Sullivan [12]). On theoretical grounds, regional therapy seems especially well adapted to treatment of retinoblastoma in view of the anatomy and blood supply of the retina. Initially, in two hopeless cases, with central nervous system invasion, TEM was introduced into the internal carotid artery on the side of the tumor under direct operative vision. The dual purpose was to assess the direct effect on the tumor, as well as the optimal dosage.

The pathologic findings are now available in the case of K. B. She had bilateral disease, and microscopic studies after enucleation of the right eye revealed extensive tumor invasion of the optic nerve. This finding has yielded a uniformly fatal prognosis. The lesion in the left eye appeared to be more favorable, and treatment was started before the pathologic report on the enucleated eye was completed. She received the standard course of x-ray treatment to the left eye and 3 doses of oral TEM. A mass in the right orbit appeared, and cells in the spinal fluid were subsequently noted. She was then treated on two successive days with a total of 5 mg. of TEM given by injection into the right internal carotid artery under direct vision. Subsequent experience has shown us that the dose of TEM in the first case was too high, and at present we are studying the effect of smaller doses. After intra-arterial TEM the white blood cell count may fall within 3 to 5 days, reaching nadir at 14 days and rising by 21 days, thus paralleling nitrogen mustard rather than slow-acting oral TEM. As a result of the high dosage in this patient, the terminal course was further complicated by aplastic anemia. During the three weeks following treatment the huge orbital mass on the injected side almost vanished. On histologic study only a hemorrhagic, necrotic residue containing some cells with deep-staining nuclei and scanty cytoplasm was found in the orbit and in the intracranial brain stem extension. This was in contrast to the findings in the left eye treated earlier with oral TEM and radiation, which showed active tumor with rosette formation.

In view of the encouraging immediate effect on the tumor and in the light of subsequent experience, we are employing single

§ References 9 and 10.

therapeutic doses of 1.5 to 2.0 mg. intra-arterially in specially selected cases. The results are far too preliminary to be reported on at present.

In summary, it must be said that TEM therapy alone has not proved curative in the treatment of any neoplasm in the past, no matter how beneficial it may appear initially. No different end-result should be expected in patients with retinoblastoma. Consequently, the use of TEM alone has not been assayed in the present series. We feel, therefore, in view of the evidence obtained in the present study, that although TEM exerts a clear-cut, beneficial effect on retinoblastoma, its present role is in a synergistic association with properly administered radiation.

CONCLUSION

As a result of our 21 months' experience with the treatment of retinoblastoma by a combination of radiation and triethylenemelamine, we feel that these two agents implement each other in such a synergistic manner that they offer the best method to date of treating this type of tumor.

Mrs. Virginia Pampellonne assisted in this work.

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Effect of Oxygen Weaning in Retrolental Fibroplasia

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The conflicting views on the role of oxygen in the pathogenesis of retrolental fibroplasia have created much confusion. Two studies made at the Philadelphia General Hospital have already been reported.* One study was based on the uncontrolled use of oxygen. The second study utilized controlled oxygen environments for premature infants with birth weights below 1.8 kg. (4 lb.). Both studies showed that the incidence and severity of retrolental fibroplasia are significantly increased by the use of greater oxygen supplements and the rapid reduction in the oxygen concentration of the infant's environment. Additional data were presented to support the hypothesis that the major precipitating factor in the disease was the improper withdrawal of supplemental oxygen. It was, however, clearly stated that the direct effects of oxygen were also of importance.

The present study was designed to evaluate the effects of the following factors:

1. Rapid and slow withdrawal of supplemental oxygen
2. Duration of administration of supplemental oxygen
3. Total amount of supplemental oxygen with a fixed maximum concentration of 50%

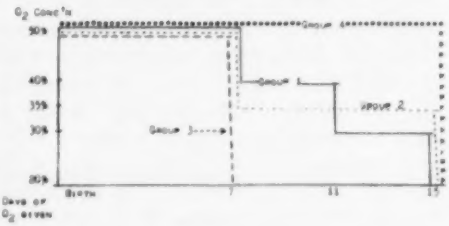
METHODS AND MATERIALS

Infants with birth weights between 1.36 and 1.8 kg. (3 and 4 lb.) were placed alternately in one of four groups to receive oxygen, as shown in Table 1.

TABLE 1.—*Duration and Concentration of Oxygen for Each Group of Infants*

Group 1	Group 2
50%..... 7 days	50%..... 7 days
40%..... 4 days	35%..... 8 days
30%..... 4 days	
Group 3	**Group 4**
50%..... 7 days	50%.....15 days

GRAPHIC REPRESENTATION OF TABLE 1.



If the rapid reduction in the oxygen concentration of an infant's environment is the major factor in the development of the disease, then the incidence of the disease should be lower in Group 1 than in Group 3. This would be true despite the fact that Group 1 received more oxygen than did Group 3.

All infants weighing less than 1.36 kg. (3 lb.) were given oxygen according to the schedule of Group 1. This would weight the study against the hypothesis that the weaning factor was of greater importance, since one would expect that the incidence of the disease would be higher in the smaller infants.

The infants were grouped on admission to the premature nursery by the pediatrics department. When the ophthalmologist was examining an infant, he was not aware of the infant's group. The fundi were examined every week for six weeks and, if normal, every two weeks thereafter until the child was discharged from the hospital. Abnormal fundi were seen every week. If an an infant showed any evidence of retrolental fibroplasia, a second ophthalmologist was called in for a confirmatory examination.

* References 1 and 2.

If a baby had the disease and it appeared to be getting worse, the infant was replaced in an oxygen-enriched atmosphere of 50% until the changes regressed or became stationary. The oxygen concentration was then gradually reduced until the infant was placed in room air.

Our results with oxygen therapy have been uniformly successful. Therefore, we did not think it proper to withhold treatment for the sake of obtaining controls.

Oxygen concentrations were checked daily and were within ±5% of the desired level.

The disease was classified under the following active stages:

Stage 1. Dilated and tortuous vessels and/or abnormal neovascularization at the ends of the retinal vessels

Stage 2. Stage 1 plus hemorrhages and edema

Stage 3. Retinal detachment

We have included the abnormal neovascularization in Stage 1, rather than Stage 2, since we have seen a number of eyes that have had neovascularization but did not have dilated or tortuous vessels.

Infants were considered to have had the disease if they showed evidence of retinopathy on two or more occasions, as seen by two different observers. This is the criterion used in our analysis.

RESULTS

The incidence of retinopathy in each of the four groups is shown in Table 2. As can be seen, the incidence of the disease increases progressively with each group. This was what we expected on the basis of conclusions from our previous studies. The chances of simply guessing such a result would be only 1 in 24. This in itself is of importance in supporting the conclusions which were drawn concerning the relation of oxygen to retinopathy of prematurity.

TABLE 2.—*Incidence of Retinopathy in Each Group*

	Group 1 (Weaned from 50% O_2)	Group 2	Group 3 (Not Weaned)	Group 4 (Not Weaned)	Total
Disease	5 (19%)	3 (30%)	9 (56%)	6 (67%)	23
No disease	21	7	7	3	38
Total	26	10	16	9	61

The incidence of the disease in Groups 1 and 3 gives the most significant data in evaluating the relative importance of the direct effects of oxygen as compared with the effects of oxygen weaning. If the direct effect of oxygen is more important, then Group 3 should have a lower incidence of the disease than Group 1, since this group received less total oxygen than did Group 1. This group also received it for less time.

As shown in Table 2, there were 26 infants who received oxygen as prescribed for Group 1; only 5 of these developed retinopathy. Nine of the 16 infants in Group 3 developed retinopathy. Analysis of the data showed that the incidence of the disease was significantly higher in Group 3 than in Group 1 (χ^2 method with Yates' correction: $P = 0.03$). The results are even more impressive when one considers that all infants below a birth weight of 1.36 kg. (3 lb.) were placed in Group 1.

The incidence of retinopathy is also significantly higher in Group 4 than in Group 1 (exact χ^2 method: $P = 0.015$).

It is important to note that none of the infants showed any evidence of the disease while receiving oxygen.

Seven infants were given oxygen therapy because their retinopathy appeared progressively worse on repeated examinations. Six were infants used in this study, while one was an infant whose original oxygen schedule was abandoned because of other factors. All these infants had a rapid and definite improvement in their fundus picture when oxygen administration was reinstituted.

During this 12-month study, there have been 30 infants with some stage of retinopathy. Twenty-three were from the controlled series, and 7 were infants who received variable amounts of oxygen. In none of these 30 infants did the disease progress to the stage of retinal detachment.

From the foregoing data, we must conclude that the improper withdrawal of oxygen supplements is a significant factor in the development of retrolental fibroplasia. The study also strongly suggests that oxygen therapy, once the disease has developed, is of value in most cases.

A comparison of Groups 3 and 4 is of interest in considering the effects of duration of oxygen administration, since Group 4 received oxygen for 15 days while Group 3

received oxygen for 7 days. The incidence of retinopathy was higher (67%) in Group 4 than in Group 3 (56%). This difference is not statistically significant, however.

In emphasizing the importance of the weaning factor in this disease, it would be easy to neglect the direct effects of oxygen. These vascular effects are of great importance, since oxygen administration predisposes to the disease in the first place. One infant whom we observed showed what we believe to be one of these effects. This infant was placed in an environment of 50% oxygen. Four days following this, the peripheral portion of all the main veins showed abrupt narrowing to less than one-half the diameter proximal to the narrowing. This narrowing continued to the distal ends of the vessels. Within a week of the time the infant's oxygen supplements were stopped, all the vessels became dilated and tortuous; marked retinal edema appeared, and a few hemorrhages were noted. This infant was replaced in an atmosphere of 50% oxygen. By the following week the retinal edema had disappeared, and the vessels were normal in size, except for a number of focal narrowings of the veins. As the concentration of the infant's oxygen environment was gradually reduced, these focal narrowings disappeared.

It is very easy to conceive that this narrowing could progress to complete closure of the ends of newly forming blood vessels. This phenomenon has been shown in animals by Ashton and Patz.† When these vascular channels start to grow again, they do so by forming extra vessels. This could be analogous to the way a tree branch grows after being pruned.

Another observation worth mentioning is the neovascularization and the compensatory dilatation of vessels at the site of a hemorrhage. One infant developed a single hemorrhage in the periphery of one eye four weeks after birth. The vessels leading to this hemorrhage became dilated within the following week but returned to normal as the hemorrhage absorbed. All the other vessels remained normal. New capillary tufts appeared at the site of the hemorrhage. Figure 1 is a flat retinal preparation of this eye and shows the findings described.

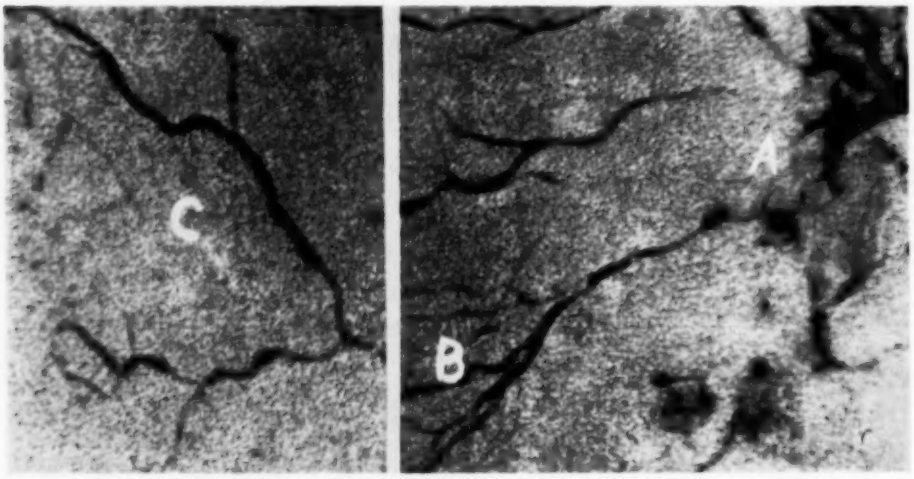


Fig 1.—Flat retinal preparation of an 8-week-old premature infant (birth weight 1.7 kg. [3¾ lb.]). Blood in vessels stained by a modification of the Pickworth benzidine method. *A* shows site of old hemorrhage in superior temporal retina with abnormal capillary ends growing toward vitreous. *B* shows abnormal ends of retinal vessels which originally were at *A*. *C* shows normal-appearing ends of inferior temporal retinal vessels of the same eye.

† References 3 and 4.

COMMENT

There are two apparently opposite points of view concerning the relationship of oxygen to retrolental fibroplasia. One is that the disease is due to a relative anoxia; the other is that the disease is primarily due to the direct effects of oxygen. Both theories require the use of supplemental oxygen.

Neither of these theories alone can explain most of the common phenomena observed in retinopathy of prematurity. Furthermore, infants who have never received supplemental oxygen have developed the disease. The most logical explanation would appear to be as follows:

Retinopathy of prematurity is the result of retinal anoxia. (Vasodilation, hemorrhages, edema, and neovascularization all are anoxic phenomena.) This anoxia may be the result of three different mechanisms. These may operate singly or in combination, as follows:

1. Supplemental oxygen causing vasoconstriction and a decreased blood supply. Rapid withdrawal of the supplemental oxygen suddenly decreases the blood oxygen level, and anoxia results because of decreased blood supply. Abnormal compensation takes place in the form of dilated and tortuous blood vessels, hemorrhages, edema, and occasionally neovascularization. Although abnormal capillary proliferation is considered to be the basic pathologic lesion of retrolental fibroplasia, the above-mentioned changes are a definite part of the clinical picture. Therefore, their presence must be considered as indicative of the disease. This appears to be the commonest sequence of events.

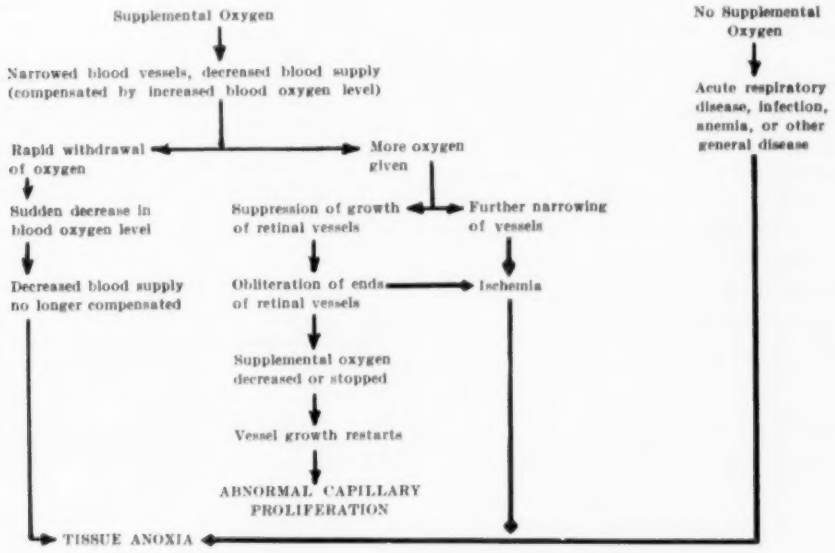

Supplemental Oxygen
No Supplemental Oxygen
Narrowed blood vessels, decreased blood supply (compensated by increased blood oxygen level)
Acute respiratory disease, infection, anemia, or other general disease
Rapid withdrawal of oxygen
More oxygen given
Sudden decrease in blood oxygen level
Suppression of growth of retinal vessels
Further narrowing of vessels
Decreased blood supply no longer compensated
Obliteration of ends of retinal vessels
Ischemia
Supplemental oxygen decreased or stopped
Vessel growth restarts
ABNORMAL CAPILLARY PROLIFERATION
TISSUE ANOXIA

Fig. 2.—Mechanisms in retinopathy of prematurity.

2. Supplemental oxygen causing vasoconstriction. Continued narrowing leads to obliteration of the ends of the newly forming retinal blood vessels and suppression of growth of developing vessels. When oxygen is stopped, the retinal vessels reopen and restart their growth. This proceeds by abnormal capillary proliferation.

It is also possible that continued narrowing and obliteration of the retinal vessels might lead to ischemia and tissue anoxia. Compensation might result in dilated and tortuous vessels with hemorrhages and retinal edema. This sequence of events is probably uncommon.

3. Systemic diseases, such as pneumonia, anemia, etc., may also result in anoxia.

These mechanisms are graphically shown in Figure 2.

Other secondary factors, such as disturbances in enzyme systems, may also play a part.

SUMMARY

A group of premature infants who were suddenly removed from an atmosphere of 50% oxygen had a significantly higher incidence of retinopathy of prematurity than did another group of premature infants who had a gradual reduction in the concentration of their oxygen environment. Infants with a progressive retinopathy were given oxygen therapy, with a definite beneficial effect.

The vasodilation and neovascularization associated with a retinal hemorrhage near the end of a developing retinal blood vessel are also described.

Three possible mechanisms by which the disease may develop are suggested.

CONCLUSIONS

1. Although retinopathy of prematurity is primarily an anoxic disease, the direct effect of oxygen is an important factor in its development. Therefore, the administration of supplemental oxygen to premature infants should be limited to the minimum amounts necessary. A 40% concentration inside the incubator is probably the level which should not be exceeded as a routine measure.

2. The commonest precipitating factor in the disease is the improper withdrawal of supplemental oxygen. Therefore, if an infant is given supplemental oxygen, a gradual reduction in the oxygen concentration of the infant's environment should be made before oxygen is stopped completely.

3. Oxygen therapy is of value after retinopathy develops if judiciously used and gradually withdrawn.

Mr. Hyman Menduke, Assistant Professor of Statistics, Jefferson Medical College, Philadelphia, assisted in the "setting up" of the experiment and the statistical analysis. The chiefs of service of the Department of Pediatrics permitted use of patients, and the residents in the premature nursery gave essential cooperation in the carrying out of this study. Mrs. Williams and Mrs. Moore, head nurses of the premature nursery, and their assistants gave invaluable help during the examination in grouping the patients and in maintaining the proper oxygen flow rates.

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Preservation of Cornea by Dehydration

A Preliminary Report

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The operation of keratoplasty is frequently limited by the unavailability of donor corneal material. This report concerns preliminary experiments on the storage of cornea by dehydration and its successful use in lamellar transplantation in animals.

Many attempts have been made to store corneal grafts over long periods, and various methods have been tried in animals, without success. Katzin (1947),[1] Leopold and Adler (1947),[2] and Smelser and Ozanics (1946) [3] all used rapid freezing in liquid nitrogen, with and without isopentane as an intermediate heat-transfer medium. In the method described in the first two papers, the grafts, after freezing, were dried in vacuo over P_2O_5 at —40 C. They were reconstituted before use, in most cases, by the addition of isotonic sodium chloride solution. Smelser and Ozanics did not dry their materials but stored them at —195 C for periods of from one hour up to five days. The grafts were then rapidly thawed by immersion in isotonic sodium chloride solution at 39 C. When thus prepared for implantation, both the frozen and the freeze-dried grafts were clear and of normal consistency. A great majority of these grafts healed well after implantation, but in most of them translucency was lost within the first three weeks. There was no lasting transparency in any of the grafts during the time they were observed.

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In 1949, Polge and associates [4] found that fowl spermatozoa treated with glycerin-saline solution retained their motility after freezing and thawing. Since that time, marked success has been reported on the survival of skin, blood, and endocrine tissue treated in a similar manner.[5] In early 1954, Eastcott and his colleagues [6] reported that human corneas treated in this way and stored at —79 C yielded lamellar grafts that differed in no way from fresh grafts in their final result and transparency.

Since success had been obtained in preserving corneas by using the glycerin-saline technique with freezing, we considered the possibility of carrying the process one step further and drying the tissue in a vacuum. This consisted essentially of drying corneas by removing the water from the glycerin solution.

METHOD

The entire cornea was removed from cats and placed in a glass container with a solution of 15% glycerin and isotonic saline. The container and contents were then cooled by immersion in a mixture of crushed solid carbon dioxide (dry ice) and alcohol. A vacuum pump was connected to the container and the water removed. The temperature of the solution was kept sufficiently low to prevent the water from boiling off too rapidly. This left the corneas in a solution of approximately 100% glycerin. Later, it was reasoned that the process could be simplified by using 80% glycerin with saline to dehydrate the corneas, since this involved removing less water. After all the water was removed and dehydration was complete, the corneas were stored in an airtight container at room temperature. Preserved in this way, they remained clear and transparent. Some corneas were preserved in 95% commercial glycerin and stored at +4 C (Table; Cats 12 and 13).

Corneal nonperforating lamellar grafts were done on cats which were anesthetized with intravenous pentobarbital (Nembutal).

At certain intervals corneas were removed and reconstituted in isotonic saline. After approximately 20 minutes they assumed their normal contour and

Results with Dehydrated Corneal Grafts

Cat No.	Storage Time of Treated Donor Graft	Size of Graft, Mm.	Status of Graft	Post-operative Age of Graft, Mo.
1	1 wk.	5.5	Clear	10
2	1 wk.	6.0	Clear	10
3	3 days	5.5	Clear	8
4	3 days	6.5	Clear	8
5	3 days	5.0	Clear	8
6	1 wk.	5.5	Clear	8
7	1 wk.	5.5	Clear	8
8	18 days	5.5	Clear	7½
9	18 days	5.5	Clear	7½
10	4 mo.	5.5	Clear	5½
11	4 mo.	7.0	Clear	5½
12	1 mo.	5.5	Nearly clear	6
13	1 mo.	5.5	Nearly clear	6

appeared white and opaque. Round lamellar grafts, ranging from 5 to 7 mm. in diameter, were then cut from the whole cornea. These donor grafts were placed on the recipient eye from which a similar-sized section of cornea had been previously removed, and were held in place by four direct silk sutures. The cats were given 150,000 units of penicillin postoperatively and the dose repeated two days later. Atropine and oxytetracycline (Terramycin) ointment were instilled daily in the eye which had been operated on.

RESULTS

The grafts were inspected frequently, and they remained white and opaque for from five to seven days, until the epithelium from the host had covered the graft. During the second week there was rapid clearing, and by the third week the grafts had reached their final transparency.

In all cases the grafts became transparent, and in many instances it was difficult to distinguish the eye with the corneal graft from the eye which had not been operated on. In Cats 12 and 13 (Table), in which the cornea grafts were preserved in only 95% glycerin, the grafts became transparent but there was a slight diffuse clouding. In no case was there any ingrowth of vessels from the host cornea into the graft. Corneas were stored for as long as 4 months, and grafts have remained clear for 10 months.

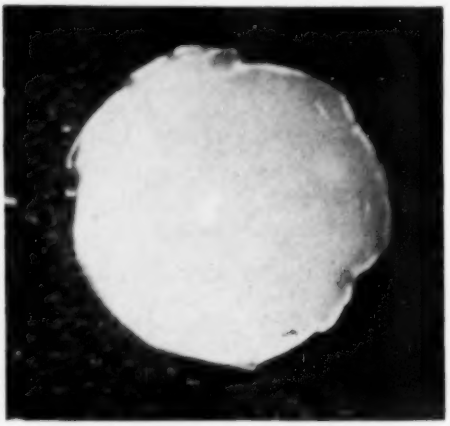

Fig. 2.—Appearance of corneas after being reconstituted in isotonic saline.

Fig. 1.—Appearance of corneas that have been dehydrated in glycerin.

Fig. 3.—Lamellar graft in right eye four months after operation. Graft previously dehydrated and stored in glycerin for two months.

COMMENT

Success has been achieved in preserving corneas through a process of dehydration. By the complete removal of water, it is felt that the cellular structure of the tissue is left undisturbed and that cellular metabolism is brought to a complete standstill. Glycerin is necessary in dehydrating the corneas, in which glycerin takes the place of the water in the tissues. Complete dehydration of corneas in a strictly aqueous solution leaves them with no cohesive structure to remain intact, and they will often powder.

The part glycerin plays in the tissues is not exactly understood. However, it is felt that the reason success was obtained in storing grafts in 15% glycerin and saline at low temperature [6] was because glycerin apparently protected the cell from the crystallization effect of the water, which, on freezing, has been reported to damage the cell. When the tissue is prepared in this manner and stored at low temperature, metabolic activity is brought to a halt without any change in the cell. Similarly, when corneal tissue is completely dehydrated in glycerin, it can be left at room temperature and will yield clear grafts after prolonged periods of storage. Corneas stored in 95% commercial glycerin for one month at +4 C (Table; Animals 12 and 13) were slow on absorbing water when reconstituted in saline and did not become white as did the completely dehydrated grafts. When grafted, they had a stormy postoperative course, and at times it appeared they might slough. The final transparency was not obtained until six weeks after healing. Since these grafts were not stored at a low enough temperature and since there remained 5% water in the solution, it is probable that they were not sufficiently protected from some autolysis.

It is felt that this method of storing corneas at room temperature may have wide application in the future in making human corneas more readily available and thus prevent the shortage and waste that exists at present.

SUMMARY

A successful method to preserve corneas for transplantation by dehydration is reported.

Corneas completely dehydrated in glycerin can be stored for an indefinite period of time and will yield clear, nonperforating lamellar grafts.

Raymond B. Gochenour, D.V.M., and Pfc. Eugene W. Plischke, of the Army Medical Service Graduate School, gave technical assistance.

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Effect of Retrolental Fibroplasia in Children

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Retrolental fibroplasia may be a lifelong disease. The child with retrolental fibroplasia may become a useful, independent adult with a handicap of some degree of ocular damage, or, in the extreme severe form of the disease, may have a futile, passive life as a physically and mentally stunted child in a state institution for retarded children. Infantile blindness is then a minor, and the total deterioration a major, catastrophe of life. Most children range between these two extremes. The progress of these children now can be determined, as sufficient time has elapsed for an investigative study.

THE STUDY

An evaluation of the effects of retrolental fibroplasia on children was made so that ophthalmologists and pediatricians may know what happens to the child in later years. For this purpose all of the children with retrolental fibroplasia of the University of Chicago Clinics were followed in the clinic, home, and institution and were examined repeatedly after they were seen during the first year of life. They were investigated by pediatricians, social workers, teachers, psychologists, and psychiatrists as a part of a study of blind children. With very few exceptions, the record on each child was complete. There was no doubt about the diagnosis of retrolental fibroplasia. It was confirmed histologically on 16 eyes of these children.

The status of the 107 children for the year of 1954 is given briefly in Table 1. The first infants with retrolental fibroplasia appeared in 1937 and are now 17 years old. The youngest children included in this report were born in 1950. The course of the younger children is not quite predictable, but it probably will not change greatly. With the birth weight, the letter T is used to indicate a sibling of the 17 multiple births. The final stage of retrolental fibroplasia is given in Roman numerals instead of letters —I is the minimal and V is the maximal damage. The upper symbol registers for the right eye and the lower for the left eye. The refraction is shown in spheres and cylinders. In the column for school the word "institution" means the child is permanently confined in the state institution for retarded children.

Age.—The children with retrolental fibroplasia who were born from 1937 to 1941 fared poorly. One-third died of disease that seemed to be unrelated to retrolental fibroplasia. The common cause of death was infection. Congenital neurological disease was frequent only in this group. There was no way to determine from the records whether extremely high concentrations of oxygen were given and, if so, whether they damaged the brain, or whether, because of congenital neurological disease, oxygen therapy was needed for survival.

Children who were born during the last 10 years were less handicapped physically and mentally. This improvement of health was in part related to better management of their lives.

Birth Weights.—The birth weights of children in groups of 500-gm. intervals were as follows:

Birth Weights, Gm.	No. of Children	Per Cent
1000 or less	13	12.1
1000 to 1500	57	53.2
1500 to 2000	29	27.1
2000 to 2500	3	2.8
Over 2500	5	4.8
Total	107	100.0

From the Division of Ophthalmology, Department of Surgery, of the University of Chicago.

This investigation was supported by the Douglas Smith Foundation for Medical Research and the Chicago Community Trust of Chicago.

The cooperation of Miss Miriam Norris of the Project for Study and Development of Preschool Blind Children has made this report possible.

TABLE 1.—*Effect of Retrolental Fibroplasia on Children*

Birth	Birth Weight, Gm.	Days in Hospital	Final Type Retrolental Fibroplasia	Disposition: Vision	Disposition: Refraction	School	Mentality	Comment
				Age 17 Years				
1937	1835	51	V V	LP 0	.....	Braille	Good	.....
1937	900	70	V V	0 0	.....	.....	Retarded	Died (1941) of brain tumor, not retinoblastoma Autopsy
1937	1040	66	V V	0 0	.....	Institution	Imbecile	Institution
1937	3410	12	V V	0 0	.....	.....	Recommended to institution	Died (1939) of pneumonia. Congenital hydrocephalus; brain edema Autopsy
				Age 16 Years				
1938	1770	21	II III	Large objects	.....	None	Retarded	Died (1946). Acute enteritis; spastic quadriplegia
1938	2840	20	III V	LP LP	—10.00	.....	Retarded	Died (1938). Marked congenital hydrocephalus
1938	2775	10	I I	LP LP	.....	.....	Recommended to institution	Died (1940). Cerebral and cerebellar agenesis, microcephaly, major convulsions
1938	705	100	V V	0 LP	.....	Braille	Dull normal	.....
				Age 15 Years				
1939	960	72	V V	LP 0	.....	Braille—8th grade in 1952	Good	.....
1939	1550	60	V V	0 0	.....	State school for blind	Retarded	Congenital motor disability
1939	810 T	32	V V	0 0	Cataracts	Braille Public school	Retarded mentally and physically	Polydactylism; retrolental fibroplasia early, rapid, severe
				Age 14 Years				
1940	1288	88	V IV	0 5/200	Nystagmus —3.00	Braille—5th grade, 1952	Dull	Partly deaf
				Age 13 Years				
1941	1745	32	V V	0 0	.....	Institution	Very low	.....
1941	1740	48	V V	0 0	.....	Institution	Very low	.....
1941	1120	110	V V	LP LP	.....	State school for blind	.....	Improving
				Age 12 Years				
1942	1431	29	V V	LP LP	.....	Braille	Retarded by home environment	.....
1942	1580	47	V V	0 0	.....	Braille	Excellent	.....
1942	1585	40	II II	8/200 10/200	—20.00 —10.00	Sight-saving school	Excellent	Foster home
1942	1360	76	V V	0 0	.....	Institution	Imbecile	I.Q. 32
1942	850	60	V V	0 LP	.....	Braille	Good	.....
1942	808	91	II II	20/200 1/200	— 5.50 —18.50 ⊃ + 3.00 x 90	Institution recommended	Retarded	.....
1942	1620	..	I	20/100	—14.00 ⊃ + 1.50 x 90	5th grade	Excellent	Premature sisters, 4 years and 6 months of age, have retrolental fibroplasia
			I	20/30	—15.00 ⊃ + 2.50 x 90	.....		
				Age 11 Years				
1943	1105	70	V II	LP LP	.....	Home	.....	Died (1943) enteritis Umbilical hernia; hemangioma of scalp; brain normal. Autopsy
1943	920 T	64	IV IV	Large objects	—25.00 —25.00	Braille	Average good	.....
1943	1875	18	I IV	20/30 10/200	—12.00 — 5.00	Sight-saving school	Excellent	Above average
1943	1428 T	54	V V	0 0	.....	Braille	Average	Once recommended to institution but now up to age level

TABLE 1.—*Effect of Retrolental Fibroplasia on Children*—Continued

Birth	Birth Weight, Gm.	Days in Hospital	Final Type Retrolental Fibroplasia	Disposition: Vision	Disposition: Refraction	School	Mentality	Comment
1943	1792 T	35	V IV	0 LP	.....	Institution	Retarded	.....
1943	1760	37	V V	LP LP	.....	Braille	Fair	.....
				Age 10 Years				
1944	1150	66	V V	0 0	.....	Braille	Fair	Mild cerebral palsy
1944	1167 T	..	V V	LP LP	.....	State school for blind	Good	.....
1944	1316 T	42	V V	LP LP	.....	Braille	Retarded	I. Q. 58
1944	1204	..	IV V	LP 0	.....	Braille	Up to age level	In foster home
1944	980	68	V V	0 0	.....	Institution	.....	Greatly retarded; environment poor
				Age 9 Years				
1945	1344 T	72	V V	0 0	.....	Braille	At age level	.....
1945	1815	39	IV V	LP 0	.....	.....	Retarded	Died (1946) Spastic quadriplegia
1945	1540	42	V III	0 15/200	..... −1.25 ⊃ −0.25 x 100	Sight-saving school	Excellent	.....
1945	2332 T	99	V V	0 0	.....	Recommended for institution	Greatly retarded	.....
1945	3220	..	V I	0 20/40	+7.50 ⊃ +2.00 x 115	Regular school	Good	With sighted children
1945	908	99	V V	0 0	.....	Recommended for institution	Very retarded	.....
1945	1304	64	II II	HM HM	.....	Recommended for institution	Greatly retarded	Cerebral hypogenesis; I. Q. 27
1945	1450	39	III III	20/70 20/70	−0.50 ⊃ +1.25 x 90 +0.50 ⊃ +1.25 x 90	Sight-saving school	Excellent	Umbilical hernia
				Age 8 Years				
1946	1458	42	V V	0 0	.....	State school for blind	Fair	In foster home
1946	1580	54	V V	0 0	.....	Braille	Up to age level Good	....
1946	1415	46	V V	0 0	.....	Braille	Good	.....
1946	1250	68	V V	0 0	.....	Institution	Retarded	Died (1952); congenital heart disease
1946	1260	73	V V	LP LP	.....	Home	Retarded in part by environment	Hemiparesis
1946	1190	68	IV IV	LP LP	.....	Institution recommended	Retarded	.....
1946	1092	81	V V	0 0	.....	In institution	Retarded physically and mentally	Congenital craniofacial deformity
1946	1405	51	IV III	LP 5/200	..... +3.00	Braille	Up to age level	.....
1946	1456	39	V V	0 0	.....	Braille	Up to age level	Excellent; plays baseball, roller skates
1946	1736	42	V V	0 LP	.....	Parochial school	Good	.....
1946	1559	65	V V	LP LP	.....	In institution	Retarded physically and mentally	Retardation in part related to parents
1946	2095	..	V V	0 0	.....	Braille	Good	.....
1946	1286	64	V V	0 0	.....	Braille	Unknown	Retarded
1946	1588	22	V V	LP 0	.....	Braille	Good	.....
				Age 7 Years				
1947	1210	62	III III	HM HM	.....	Sighted nursery	Good	.....

* This child's record is inserted for comparison only.

TABLE 1.—*Effect of Retrolental Fibroplasia on Children*—Continued

Birth	Birth Weight, Gm.	Days in Hospital	Final Type Retrolental Fibroplasia	Disposition: Vision	Disposition: Refraction	School	Mentality	Comment
1947	1280	64	V	LP	.....	Sighted nursery	Up to age level	.....
			V	LP				
1947	1715	38	I	20/100	−7.00	Sight-saving school	Good	Hernias
			I	20/70	−5.00			
1947	1305	51	IV	LP	.....	Braille	Retarded	Environment poor
			V	0				
1947	1502	29	V	LP	.....	Braille	Excellent	.....
			V	0				
1947	1080 T	62	V	0	.....	Nursery	Good	Spastic diplegia
			V	LP				
1947	1210	72	II	20/200	−10.00	Unknown	Good	.....
			II	20/200	−12.00			
1947	1620 T	21	V	0	.....	Braille	Good	Was very retarded
			V	0				
1947	1530	39	V	0	.....	Braille	Up to age level	Good
			IV	Large objects				
					Age 6 Years			
1948	1170 T	63	V	0	.....	Sighted nursery	Good	Slight hemiparesis
			V	0				
1948	1300 T	63	IV	1/200				
			IV	5/200	.....	Sighted nursery	Excellent	.....
1948	1021	..	V	0				
			V	0	.....	Institution	Retarded	Omphalocele; mild hemiparesis
1948	1115 T	77	III	5/200	−3.00 ⌒ +1.00 x 90		Good	.....
			III	5/200	−4.00 ⌒ +2.00 x 90	Nursery		
1948	1360	28	V	0				
			V	0	.....	Unknown	Good	.....
1948	1100	59	V	LP				
			V	LP	.....	.....	Fair	Died (1962); acute nephritis
1948	907	72	V	0	.....	Braille		
			V	0	.....	Sighted nursery	Good	.....
1948	1350	43	III	20/50	0			
			III	20/50	−0.500	Sight-saving school	Good	In foster home
1948	1810	60	V	0			Fair	In foster home; bilateral calcaneovalgus
			V	0	.....	None		
1948	1075	59	III	20/70	−20.00 ⌒ +6.25 x 90			
			III	20/50	−11.00 ⌒ +1.75 x 95	Sighted nursery	Good	.....
1948	1078	68	V	0	.....	.....	Retarded	Died (1948), cortical toxic encephalopathy. Autopsy
			V	0				
					Age 5 Years			
1949	1255	88	I	6/20	−6.00 ⌒ −1.00 x 180			
			I	6/20	−5.25 ⌒ −1.00 x 150	Nursery	Good	.....
1949	1142	120	V	0				
			V	0	.....	Nursery	Good	.....
1949	1021 T	..	V	0	.....	State school for blind	Good	.....
			V	0				
1949	1060	87	I	20/100	−11.00 ⌒ −2.00 x 180			
			I	20/100	−11.00 ⌒ − 1.00 x 180	Sight-saving school	Good	.....
1949	1540	44	V	0				
			V	0	.....	Braille	Excellent	Top in school, high I. Q.
1949	1175	94	II	Large objects			Good	Not seen for two years
			II	Small objects		Unknown		
1949	1295	23	V	LP				
			V	0	.....	Unknown	Good	Foster home
1949	1430	47	I	Small objects				
			I	Small objects		Home	Good	Untraceable
1949	865	98	III	Small objects		Sighted nursery	Slow mentally	Spastic
			III	Small objects				
1949	1283	66	V	LP				
			V	LP	.....	Crippled school	Good	Double hemiparesis
1949	1620	41	III	20/200	−1.25			
			III	20/200	−1.25	Sighted nursery	Good	Foster home; adopted
1949	1581	48	I	20/200	−16.00			
			V	0	.....	Sighted nursery	Poor	Improving
1949	2160	21	III	20/100				
			III	20/100	.....	Sighted nursery	Excellent	.....
1949	1455	43	V	0				
			V	LP	.....	Braille	Fair	.....
1949	1417	58	V	0			Good	Retarded by home environment
			IV	LP	.....	None		

TABLE 1.—*Effect of Retrolental Fibroplasia on Children*—Continued

Birth	Birth Weight, Gm.	Days in Hospital	Final Type Retrolental Fibroplasia	Disposition: Vision	Disposition: Refraction	School	Mentality	Comment
				Age 4 Years				
1950	992	63	V V	0 0	.....	Braille	Good	.....
1950	1460	56	V V	0 0	.....	Sighted nursery	Good	.....
1950	1219	..	III	10/200	−6.00 ⌒ −1.00 x 90			
			III	5/200	−6.00	Regular school	Good	.....
1950	1474	38	V V	LP LP	.....	Sighted nursery	Good	.....
1950	907	..	III V	HM LP	−1.50	None	Good	.....
1950	1172 T	39	II I	HM 20/50	+8.00 ⌒ +2.50 x 75	Nursery	Good	.....
1950	1651 T	39	II II	HM HM	+10.00 + 9.00	Nursery	Good	.....
1950	1995	38	V V	0 0	.....	Sighted nursery	Good	.....
1950	1276	57	V V	0 0	.....	Kindergarten for blind	Good	.....
1950	1021	..	IV V	LP 0	.....	None	In part retarded by environment	.....
1950	1460	40	IV V	LP 0	.....	Braille	Good	.....
1950	1616	52	V V	0 LP	.....	Braille	Good	.....
1950	1304	25	V V	0 0	.....	Sighted nursery	Good	.....
				Age 3 Years				
1951	1360 T	12	V V	LP 0	.....	Sighted nursery	Poor	Physically stunted; delicate health
1951	1790 T	35	I	Small objects	−14.50 ⌒ +50 x 90	Regular school		
			I	...	−13.50 ⌒ +50 x 90	Vision good	Good	Triplet to above
1951	1725 T*	37	No RLF	20/30+ 20/30+	.....	Regular school	Excellent	Given much oxygen in hospital; 37 days; triplet to above
1951	1531	46	V V	LP LP	.....	None	Fair	Foster home; early hypotonia; now good

* This child's record is inserted for comparison only.

About 10% of the children were in the previable group. The high percentage was probably related to a high survival rate. About 5% had a birth weight of over 2500 gm. These infants were not regarded as premature.

Days in Hospital.—The time the infant was in the hospital is some indication of the difficulty of survival. About 13% of the children were in the hospital 10 to 30 days; 70%, from 30 to 70 days, and 17%, from 70 to 110 days. Those who were in the shortest and longest times had a high rate of mental and physical retardation. The previable infants who were kept in the hospital a long time commonly had a severe type of retrolental fibroplasia.

Stages of Retrolental Fibroplasia.—In Table 2, the stage of the healed form of retrolental fibroplasia is given for the better eye. The stage of the disease was not closely related to the degree of blindness. About 35% of the children in Stages II and IV had some useful practical vision besides light perception. A very few in Stage I had sufficient vision to attend regular school.

Included in Table 2 is one family in which there were three consecutive premature infants treated by excessive oxygen therapy ending in regressed retrolental fibroplasia; the first child, age 12, had high myopia; the second child, age 4, a macular dystrophy, and the third child, age 6 months, retinal stalks. The child with a milder form of

retrolental fibroplasia was not expected to have the visual and environmental problems of the children which have been presented in Table 1.

Vision.—The range of the corrected vision of the five healed stages of retrolental fibroplasia is given in Table 2. Various factors, such as fixation nystagmus, high optical correction, and mental retardation, decreased vision. In a few children binocular vision was remarkably higher than vision of either eye. The numerical visual evaluation was often lower than that found in practical tests. The past experience of the child was a great aid in the interpretation of what he saw, even when the visual acuity was low.

TABLE 2.—*Vision in Retrolental Fibroplasia*

Stage of Retrolental Fibroplasia	No. of Children	Corrected Vision
I	11	20/30 to 20/200
II	8	10/200 to H. M.
III	14	20/50 to H. M.
IV	12	5/200 to L. P.
V	62	L. P. to nil

The vision in Stage I varied greatly. The damage to the fundus corresponded with the visual acuity. The picture was not uniform. Anomalous vessels, generalized retinal atrophy, delayed myelination of the optic nerve, retinal dystrophy, and macular dystrophy were some of the defects. The vision in the other advanced stages of retrolental fibroplasia more closely matched the structural type of lesion.

Refraction.—The range of refractive error by retinoscopy varied from —25 to +10 D. spheres and 6.25 D. cylinders. About 60% of the children had a myopia over 5 D. and 40% over 10 D.

School.—The age group distribution of school children is shown in Table 3. The group from 13 to 17 years of age was limited by the early deaths. The percentage of mental retardation particularly associated with physical defects and low weight was high. This, in turn, lead to the recommendation of admission to a state institution for retarded children. The percentage of normal children with blindness was very low.

The second group, from 8 to 12 years of age, showed better physical and mental development. Their growth and environment were adjusted earlier in life than in the first group. If possible, they were kept with sighted children of the same age group. They entered school early in spite of handicaps. The teaching of Braille at home, school, or at the State School for the Blind was not delayed. When no school was available, the home environment poor, the child isolated, or when the child had incurable handicaps, the tendency was toward general retardation.

The last group had the highest educational recovery value. Those who are classified as having excellent mentality were rated above the average normal blind child. It was expected that these children would maintain their adjustment to school.

COMMENT

Vision.—In most children the vision was severely affected, particularly in the first five-year group. If there was no ocular reaction to the focused light of a standard two-cell flashlight at 1 ft. (30 cm.), the vision was recorded as no light perception. Intense lights often gave a false reaction to localized heat.

Retinoscopy, if possible, was done yearly. When sedation was needed for examination, pentobarbital (Nembutal) by rectum was given. If the child was uncooperative, gen-

TABLE 3.—*Educational Status of Children with Retrolental Fibroplasia*

Range of Birth Years	No. of Children	School					Mentality					Physical or Neurological Defect	Low Weight
		Sight-Saving Regular School	Nursery with Sighted Children	Braille	State Institution	Home	Excellent	Average	Poor	Very Low	Dead		
1937-1941	15	..	..	7	5	3	..	3	7	5	5	10	10
1942-1946	40	7	..	19	11	3	6	17	7	10	3	7	11
1947-1951	52	5	24	12	1	10	4	41	6	1	1	7	12

eral anesthesia was given. If the results from a refractive measurement actually made a definite improvement in vision, especially for distance in myopia, a full correction was given for children over 8 months of age. In children with no accommodation and with fixed pupils, the most useful lenses were tried. In high myopia, the prescription was adjusted for vertex distance. Not all children were myopic, for some had a high hyperopia. In some children the correction of the refractive error made a marked social difference in life. In severe ametropia the eyes frequently were straight if the vision was low and equal in the two eyes. Vision greater than 20/200 was often associated with squint and fixation nystagmus. A very few children who had low vision were unable to find a practical use for it because of mental retardation.

Vision was not always static. For example, in one child the eye had a partly detached retina and a solid dark cyclitic membrane behind the pupil. Two years afterward a diagnosis of retrolental fibroplasia, Stage V, was made. At the age of 7 years the vision was 10/200, and the regression was such that the fundus was well seen and a retinal stalk with septa was present.

During the recent years have appeared the effects of the milder form of retrolental fibroplasia, which have not been generally observed. The mild form of the disease became evident as myopia, amblyopia, anomalous retinal vascularity, or strabismus near school age. The corrected vision varied from 20/15 to 20/70.

Education.—The education of children with retrolental fibroplasia is a major problem. It is much more complex than the education of a child who has lost his sight later in childhood. In the severe form of blindness the child tends to have an abnormal environment of either overprotection or rejection. The lack of care and stimulation retard the child physically and mentally. Stimulation and companionship of other children are great factors in good preparation for school. Only a few of the older children with good residual sight in our series had attended regular schools. In the metropolitan area of Chicago it is usually possible for the parents to send a blind child to a sighted nursery school. The experience of being with sighted children and being accepted by them is important for later life and helps overcome the mannerisms and emotional patterns of the blind child. Education is not only by the school but by the other children.

In the early years of retrolental fibroplasia, from 1937 to 1941, the infant was considered to be a severely damaged child with an exceedingly doubtful future. The future of the child was placed in the hands of the uninstructed parents. Now the child is taken as an average blind child and treated as a normal child until proved to be otherwise. Frequently the child who had a good future was not permitted to develop and became retarded physically and mentally. Of course, some infants, in their fight for survival, had added handicaps which prevented recovery. These events may explain in part why so many were retarded in the first 10 years, from 1937 to 1946.

Most of the children, when they became older, were instructed in reading Braille in city schools or in a state institution for the blind. A few were exceptionable students with high grades in school. The average child fared better in the city school than in an institution for the blind. Once established in school, the child tended to continue. If no school was available, education was hindered, and the child was more of an emotional and social problem. The environment at home was one of the determining factors in education.

Some children were retarded physically and mentally and were placed in a state institution for mentally retarded children, where they became irreversibly deteriorated. Only after much experience was it learned that some of these children had a recovery value, and placement in state institution was delayed until it was found that the condition of the infant was unchangeable.

Mortality.—The death rate of these children with retrolental fibroplasia was surprisingly high—8.5%. If the first 50 of the older children are taken according to age, the ratio was 16%. In the last 50 children the death rate was low—2%. There is no reasonable explanation for such a difference, for if the last 50 were followed for the same length of time, the rate would still be low.

Most deaths resulted from intercurrent infection and neurological disease. Postmortem examinations showed no pathological disease that was related to retrolental fibroplasia. Mineralogical section of the teeth gave no suggestion of a congenital disease.

Neurological Aspects.—A few infants in the hospital who survived only a few days under oxygen therapy presumably would have had a chance to develop retrolental fibroplasia if they had lived. These are not recorded as cases of retrolental fibroplasia. A certain number of infants with anatomical and physiological defects, or with birth trauma, such as anemia and cerebral hemorrhage, were given oxygen for survival. In this group were the previable infants below 1000 gm. birth weight and the infant whose respiratory mechanism was deficient. Many of these children would not have been normal even if they had survived. It was not unexpected, then, that some children with retrolental fibroplasia would be abnormal.

It was not unusual that a child with retrolental fibroplasia was stunted physically. The young child was frequently hypotonic. The musculature was flabby and undeveloped. The height and weight were less than average for the age. Many times the child was the much smaller twin or was smaller than a younger sibling. This was particularly true if the child lacked years of stimulation. A blind child tended to develop his full weight and height if he had a normal activity. In passive infants delayed motor coordination was usual. Hemiparesis, bilateral spasticity, and foot drop were observed in younger children. Some children with sufficient stimulation who were no longer restricted later overcame their marked spasticity completely.

Electroencephalograms of infants and older children with and without severe mental retardation with retrolental fibroplasia showed no waves that were characteristic for the disease. Atypical waves were not uncommon, but they gave no indication of the specific kind of brain injury.

Postmortem examination of the brains of children with retrolental fibroplasia showed no abnormal lesions that were characteristic for the disease. The common histological technique for brain sections does not necessarily furnish evidence of brain damage. Special methods should be employed to determine the effects of anoxia and unusual vascularity. The rapid growth of the brain in childhood tends to obliterate or conceal any damage that occurs from overexposure to oxygen in early infancy. Although there was no histological proof for brain injury in acute retrolental fibroplasia, it would not be surprising if it were discovered by a later investigation.

SUMMARY

Of 107 children from 4 to 17 years of age with retrolental fibroplasia who were followed until the present time, 78 attended some kind of school, 16 were at home, 17 were in a state institution for retarded children, and 9 had died. The mentality was considered good in 71, poor in 20, and very low in 16 children. Physical or neurological defects were present in 24 children, and low weight or stunted growth was present sometime during life in 33 children.

A healthy infant, although blind, through proper stimulation and environment was commonly capable of becoming a useful blind adult. The factors which retarded the child temporarily or permanently were residual damage from birth, congenital defects, and lack of proper stimulation and environment. Some infants who had a good developmental future were lost to institutions because the social conditions there for advancement of the child were not suitable.

Intermediate Sex-Linked Retinitis Pigmentosa

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Recent advances in the therapeutic armamentarium of the ophthalmologist have simplified a number of problems concerned with ocular infection and inflammation. There remains, however, an increasing incidence of developmental, metabolic, and involutional disease to challenge the ophthalmic physician. It is becoming evident that the role of the gene looms ever more significant in the etiology of a large proportion of these latter entities. It is apparent, therefore, that the ability to recognize the "carrier states" of gene-determined pathological ocular traits is most important both from the diagnostic and from the prognostic viewpoint. This study resulted from the recognition by one of us (R. L. W.) of the female carrier state of intermediate sex-linked "choroidoretinal" degeneration (a tapetal-like retinal luster) during the course of a routine refraction being performed in the ophthalmological outpatient service of the Wayne County General Hospital.

It is proposed to present herein an additional family which exhibits intermediate sex-linked retinitis pigmentosa (choroidoretinal degeneration) in which the heterozygous females (carriers) may be identified by observing ophthalmoscopically the previously mentioned tapetal-like retinal luster. This unusual retinal phenomenon has been previously described by Frost (1902),* Mann (1937),[1] Falls and Cotterman (1948),[2] and Sorsby (1951).[3]

PEDIGREE

This family constitutes Kindred 4119 in the files of the University of Michigan Heredity Clinic. The pedigree presented in Figure 1 depicts 49 relatives of the propositus, of whom 7 were examined in the Ophthalmic Clinic of the Wayne County General Hospital and in the laboratory of the Kresge Eye Institute, in Detroit. In the following section of this paper each individual member of the family will be identified by means of a double number, the first number (Roman numeral) designating the generation and the second (arabic numeral) designating the order, from left to right, occupied in the generation. The propositus, or original patient, thus becomes III-33 and her father II-8.

The propositus and her family reside in southeastern Michigan, but the majority of her other relatives live in northwestern Minnesota. Unfortunately, geographic distribution prevented more extensive study of this most interesting family.

SUMMARY OF OCULAR EXAMINATIONS

Every available member of the family was carefully examined to identify and describe the retinal pathology, as well as to rule out other ocular anomalies. With regard to the ocular findings of the more distant relatives we have been forced to accept evidence based on nonprofessional observations.

III-33 (Propositus).—This 14-year-old white girl was first examined on Nov. 13, 1953. The patient had worn glasses from the age of 9 to 11, but not since that time. She had no ocular complaints.

External Examination.—Her uncorrected visual acuity was right eye 20/50, left eye 20/40+3.

* Frost, quoted by Arnold Sorsby in personal communication.

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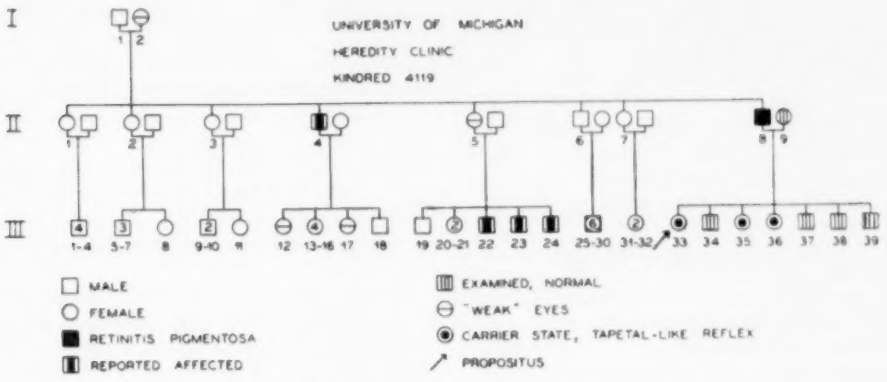

Fig. 1.—Pedigree of the F family.

The external examination revealed no ocular pathology.

Biomicroscopy of the anterior segment of each eye was normal.

Ophthalmoscopic Examination.—The vitreous, disc, and retinal vasculature were normal in both eyes.

The macular area of each eye presented a satisfactory foveal reflex.

In both eyes the immediate perifoveal macular area exhibited a tremendous deposition of golden, glistening, granular sediment which ramified into the surrounding retina in a spider-web fashion, becoming less conspicuous as the retinal periphery was approached. The golden, glistening, granuloid deposition became more difficult to visualize in the periphery due to scanty pigmentation and resultant baring of the choroidal vasculature.

Tonometric Reading.—The tonometer tension in both eyes was 23 mm. of mercury (new Schiøtz).

Cycloplegic Refractions.—Refraction gave the following correction:

Right eye: —1.00 sph ⌒ +2.00 cyl, axis 105 = 20/20—3

Left eye: —0.75 sph ⌒ +0.75 cyl, axis 80 = 20/20—1

Color Vision.—Ishihara chart tests were normal in both eyes.

Visual Fields.—Normal perimetric and central fields were demonstrated (isopters 3/330 white and 1/1000 white).

Other Tests.—Blood Kahn—negative.

Blood type—O N R_1r Fy^a.

II-8.—This 43-year-old white man, the father of the propositus, was first examined on Nov. 19, 1953. The patient had worn glasses since the age of 12. A distinct deficiency in his night vision had been first noted at the age of 16. During the past five years the patient had observed progressive loss of visual acuity and constriction of his peripheral fields.

External Examination.—The patient's uncorrected visual acuity was 6/200 in the right eye and 4/200 in the left eye. The corrected visual acuity was 2/100 in each eye. The external examination was essentially normal except for a mild periodic alternating divergent strabismus, bilateral pingueculae, and arcus senilis.

Biomicroscopy of the anterior segment was normal except for moderate posterior subcapsular cataract and scattered white minute opacities in each lens.

Ophthalmoscopic Examination.—Extensive vitreous opacities were present in each eye. The optic nerve heads were small, pale, and gliotic. The retinal vasculature was attenuated and thread-like. The macular area of each retina presented a moth-eaten, grayish infiltration, as well as conspicuous gliosis. The intermediate retina presented numerous bone-corpuscular-like strands and clumps of pigment. The smaller retinal vessels, especially the venules, exhibited perivascular accumulations of pigment. The extreme retinal periphery disclosed an increased amount, as well as clumping, of the pigment. The entire retina was conspicuously atrophic.

Tonometer Reading.—Tonometric tension was right eye 24.6 mm. and left eye 23 mm. of mercury (new Schiøtz).

Cycloplegic Refraction.—Refraction gave the following correction:

Right eye: —5.25 sph ⌒ +0.75 cyl, axis 165 = 20/100

Left eye: —5.50 sph ⌒ +0.75 cyl, axis 35 = 20/100

Maddox rod measurements revealed 12△ of exophoria and 2△ of left hyperphoria for distance and 24△ of exophoria and 1△ of left hyperphoria at 33 cm.

Color Vision.—The patient could identify none of the color testing cards (Ishihara Seventh Edition). No specific conclusion could be drawn from this finding, particularly in view of the patient's constricted visual fields and impaired visual acuity.

Visual Fields.—Bjerrum screen visual fields were constricted to 5 degrees of the fixation point in each eye (5 mm. white test object at 1000 mm.).

Other Tests.—Blood Kahn—negative.

Blood type—O MN R_1r Fy^a.

II-9.—The 45-year-old mother of the propositus was first seen on Nov. 19, 1953. This patient had no ocular complaints. Her ophthalmic examination was entirely within normal limits.

Blood Kahn—negative.

Blood type—O MN R_1r Fy^a.

III-34.—The 13-year-old brother of the propositus was first seen on Nov. 19, 1953. The patient had no visual complaints. The ophthalmic examination was within normal limits except for an area of old inactive chorioretinitis in the left eye 1 disc diameter superior and temporal to the optic disc.

Color Vision.—Ishihara chart tests were normal in both eyes.

Visual Fields.—Normal perimetric and Bjerrum screen fields were demonstrated (3/330 white and 1/1000 white test objects).

Other Tests.—Blood Kahn—negative.

Blood type—O MN R_1r Fy^a.

III-35.—This 11-year-old sister of the propositus was seen for complete work-up on Nov. 13, 1953. The patient had worn glasses since the age of 8 and had no specific ocular complaints.

External Examination.—The uncorrected visual acuity was right eye 20/50, left eye 20/200. The corrected visual acuity was right eye 20/30+2, left eye 20/30—2.

The external examination was within normal limits in both eyes with the exception of a bilateral inferior follicular hypertrophy of the tarsal conjunctiva.

Biomicroscopy of the anterior segment confirmed the follicular hypertrophy of the conjunctiva and was otherwise normal.

Ophthalmoscopic Examination.—The vitreous, discs, and retinal vasculature were normal in both eyes. The entire posterior pole of the fundus of each eye showed a peculiar golden, glistening, dust-like, striate deposition deep to the retinal vessels, which was more conspicuous at and about the macular area. These opacities became less numerous and faded out as the periphery was approached.

Cycloplegic Refraction.—Refraction gave the following correction:

Right eye: —2.50 sph ⁐ +2.50 cyl, axis 125=20/30+2

Left eye: —4.50 sph ⁐ +2.50 cyl, axis 45=20/30+3

Tonometric Examination.—Not done, but finger tension was normal in both eyes.

Color Vision.—Ishihara chart tests were normal in both eyes.

Visual Fields.—Normal perimetric and central fields were demonstrated (3/330 white and 1/1000 white test objects).

Other Tests.—Kahn test—negative.

Blood type—O MN R_1r Fy^a.

III-36.—This 10-year-old sister of the propositus was seen for complete examination on Nov. 27, 1953. The patient had worn glasses since the age of 6. She had no specific ocular complaints except for poor visual acuity in her right eye.

External Examination.—The patient's uncorrected visual acuity was right eye 7/200, left eye 20/50. The corrected visual acuity was right eye 7/200 and left eye 20/30—1. A right exotropia was present. Maddox rod measurements disclosed 35△ of exotropia and 6△ of right hypertropia at distance and 10△ of exotropia and 4△ of right hypertropia at 33 cm. The right eye was obviously the larger of the two, and Hertel exophthalmometer readings at base line 99 mm. were right eye 20 mm. and left eye 15.5 mm.

Biomicroscopy of the anterior segment of each eye was normal.

Ophthalmoscopic Examination.—The right eye showed a number of strand-like vitreous opacities. The disc was normal, and a temporal crescent was present. The vessels were normal. The macular area was devoid of pigment but showed a good foveal reflex. Conspicuous about the posterior pole were a myriad of golden, granular spots, which faded toward the periphery. The sclera showed through a very deficient choroid. The left eye revealed a normal vitreous, disc, macular area, and retinal vasculature. Situs inversus vasorum of the disc was present and a nasal conus was noted. Extending from the macular area to the periphery in decreasing concentration was a golden, glistening, striated, granular deposition deep to the retinal vasculature. The retinal pigmentation was scant throughout the entire eye. A golden, glistening pupillary reflex was particularly prominent on direct illumination of this girl's eyes.

Tonometric Examination.—Tonometric readings were not done, but finger tension was normal in each eye.

Cycloplegic Refraction.—The refraction findings were as follows:

Right eye: —15.50 sph ⁐ +1.00 cyl, axis 90=7/200

Left eye: —2.00 sph ⁐ +2.25 cyl, axis 85=20/30+2

Color Vision.—Ishihara charts were identified correctly with each eye.

Visual Fields.—Normal perimetric and Bjerrum screen fields were demonstrated for 3/330 white and 1/1000 white isopters.

Other Tests.—Kahn test—negative.

Blood type—O MN R_1R_1 Fy^a.

III-37.—This 5-year-old brother of the propositus had no ocular complaints. The ophthalmic examination was entirely within normal limits on Dec. 3, 1953.

Blood Kahn—negative.

Blood type—O M R_1r Fy^a.

III-38.—This 3-year-old brother of the propositus had no ocular complaints. His limited ocular examination presented no gross pathology on Dec. 3, 1953.

Blood Kahn—negative.

Blood type—O M R_1r Fy^a.

III-39.—This 8-month-old brother of the propositus showed no external ocular or ophthalmoscopic abnormality at the time of his limited examination on Dec. 3, 1953.

ADDITIONAL DATA AS OBTAINED BY THE FATHER OF THE PROPOSITUS

During the summer of 1954 the father (II-8) of the propositus saw several members of his family during a visit to Minnesota. He obtained the following information:

Sister II-5 was seen and had normal vision.

Son III-19 (age 28) reported normal vision.

Son III-22 (age 24) had poor night vision.

Son III-23 (age 18) had poor night vision.

Son III-24 (age 16) reported poor night vision.

Daughter III-20 (age 26) reported normal vision.

Daughter III-21 (age 11) reported normal vision.

Sister II-1 was seen and had normal vision, as did her four sons.

Sister II-7 was seen and also had normal vision, as did her two daughters.

MODE OF INHERITANCE

The distribution of cases of retinitis pigmentosa in the males and of the tapetal reflex in the females in this family suggests the presence of a sex-linked gene which produces both effects. As anticipated of an x-borne gene, the observed carrier females are the offspring of an affected father; his sons are all normal. Furthermore, all reportedly affected males in the third generation are the offspring of a sister of the father of the examined carrier females. No affected male, moreover, is the offspring of an affected father. In respect to this latter point it must be admitted that the data are indeed insufficient. An alternative genetic hypothesis, that of an autosomal gene producing different phenotypes in the two sexes, must be mentioned. This need not warrant serious consideration in that no male offspring of patients manifesting clinical retinitis pigmentosa exhibited retinal degeneration.

We have concluded (utilizing, unfortunately, considerable unsubstantiated and indirect evidence) that in this family the gene is located on the nonhomologous portion of the x-chromosome and produces clinically recognizable different ophthalmoscopic retinal manifestations in the two sexes.

RESULTS OF TESTS TO DETERMINE THE STATUS OF RETINAL PHYSIOLOGY IN CARRIER FEMALES

It is assumed that the tapetal-like ophthalmoscopic reflex present in the heterozygous female carriers is an anatomic resultant of the presence of the pathologic intermediate sex-linked gene. The usual clinical ocular examination of these patients failed to reveal any functional manifestation of the presence of this gene. It was felt that more critical and discriminating tests should be performed to investigate whether any aberration of retinal physiology existed in the female heterozygotes. These tests were carried out at the Kresge Eye Institute, in Detroit, where Dr. Elek Ludvig aided in their interpretation.

The following studies were performed on III-33, III-35, and III-36:

1. Dark adaptation
2. Light difference sense
3. Critical flicker frequency
4. Stereopsis (Verhoeff stereoptor)

1. *Dark Adaptation.*—This study revealed each of these three patients to have normal rates of dark adaptation. The average curve

appearing on each graph was obtained from three normal subjects by the method of sliding averages.

2. Light Difference Sense.—The study was performed on an apparatus so constructed that a spot of light of variable intensity appeared at random intervals on a screen of constant intensity of illumination. This spot of light subtended an arc of 10 degrees at the level of the patient's eye. The subject had merely to state whether the light was on or off. The fovea and contingent areas of the retina 2, 10, and 20 degrees from the fovea were tested in both eyes of III-33 and III-35 and in the left eye of III-36. No definite abnormality in the light difference sense was demonstrated in any of these subjects. The light difference sense in the retinal areas 10 and 20 degrees from the fovea in the left eye of III-36 was interpreted as being at the upper limit of normal.

3. Critical Flicker Frequency.—This test was assayed at the fovea and at 1, 2, 5, 10, and 20 degrees from the fovea in both eyes of III-33 and III-35 and in the left eye of III-36. Only the foveal area of the amblyopic

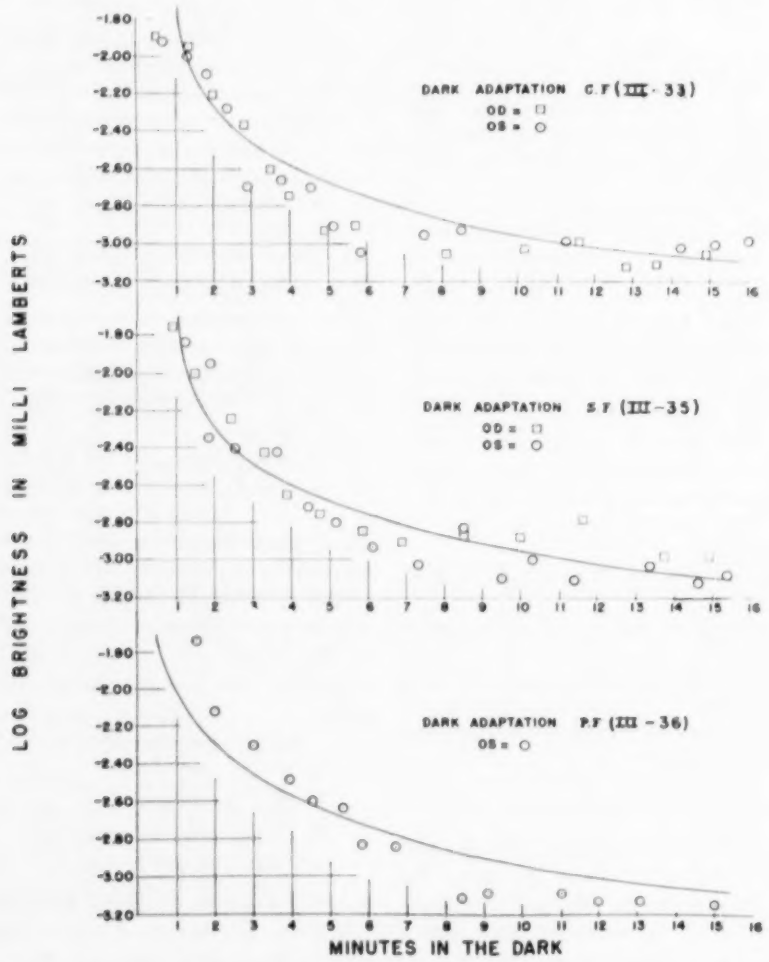
DARK ADAPTATION C.F (III - 33)
OD = □
OS = ○
DARK ADAPTATION S.F (III-35)
OD = □
OS = ○
DARK ADAPTATION P.F (III - 36)
OS = ○
LOG BRIGHTNESS IN MILLI LAMBERTS
MINUTES IN THE DARK

Fig. 2.—Log curves of dark adaptation studies of three female (heterozygote) carriers.

eye of III-36 could be satisfactorily tested. The measurements obtained in the central retinal area of each child were interpreted as being within the normal range of critical flicker frequency. The recordings made of the retina surrounding the fovea of each patient, however, were too variable to permit reliable conclusions other than that there was no gross diminution of critical flicker frequency in any of the three girls.

4. *Verhoeff Stereoptor.*—III-33 and III-35 demonstrated stereopsis within the range of normal with this instrument; III-36, as anticipated, had no stereopsis.

COMMENT

The importance of the recognition of a tapetal-like fundus reflex present in female carriers is evident both from a diagnostic and from a prognostic viewpoint. The genetically alert physician will know immediately that such a reflex indicates that the female is the heterozygous carrier of the gene for retinitis pigmentosa (male state). This knowledge should stimulate the physician to examine the remaining members of the available family and to construct a pedigree. Armed with this information, he can reliably postulate that each successive pregnancy of the "carrier" mother carries with it the potentiality of 50% normal male offspring, 50% affected male offspring, 50% carrier (heterozygous) female offspring, and 50% normal female offspring. Of course, these ratios are subject to the whims of chance, particularly in the small families of modern times. Furthermore, the physician can inform the family that all of the female offspring of an affected male will be carriers of the pathologic gene and that all of his male offspring will be normal for the trait.

It is our hope that ophthalmologists will be stimulated to examine carefully all of the close relatives of patients having retinitis pigmentosa or its allied states even though these relatives report normal visual functions.

SUMMARY

A family presenting intermediate sex-linked retinitis pigmentosa is reported. This family study was instigated by the ophthalmoscopic discovery of the tapetal-like retinal reflex in female offspring of a male affected with retinitis pigmentosa.

The routine clinical tests of visual function failed to disclose abnormality of retinal physiology in the heterozygous carriers of intermediate sex-linked retinitis pigmentosa.

Studies of dark adaptation, light difference sense, critical flicker frequency, and stereopsis likewise failed to reveal evidence of abnormality in the visual physiology of the heterozygous carriers.

Recognition of the tapetal-like ophthalmoscopic reflex will enable the ophthalmologist to draw conclusions of diagnostic and prognostic value with regard to the presence and mechanism of transmission of retinitis pigmentosa in a given family.

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Rare Form of Hereditary Epithelial Dystrophy

Genetic, Clinical, and Pathologic Study

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and
L. BYERLY HOLT, M.D., Winston-Salem, N. C.

During the year 1952 three patients—a 62-year-old man, a 66-year-old man, and a 4-year-old boy—presented themselves in the office of one of us (L. B. H.). The first needed a certificate for Blind Assistance; the second wanted a prescription for glasses, and the child was brought in because of a slight injury. All were active and in good health. None was concerned with the unusual condition of the cornea with which they were found to be afflicted. Inquiry revealed that all had common ancestors and relatives who were known to have or to have had poor vision. After examining several patients of this

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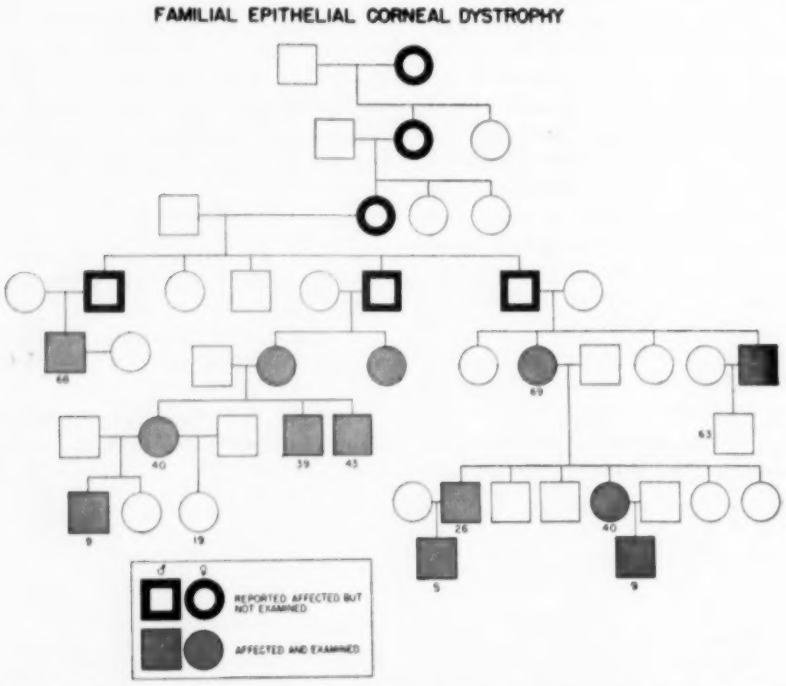


Fig. 1.—Pedigree comprising four living generations with ancestors in four generations reported to have been diseased. Simple dominant heredity is evident.

group, we initiated an investigation in two ways. First, by examining as many members of the family group as possible and getting information on former generations from all available sources, a pedigree was established. Second, a careful clinical study and a histologic examination of specimens obtained through therapeutic keratoplasty were conducted.

The genetic investigation revealed the corneal disease was prevalent in a group of people who were direct descendants of Moravian settlers in the area of Winston-Salem, N. C. The Moravians, a Protestant religious sect, came from an area near Dresden in Saxony, East Germany, and first settled at Bethlehem, Pa., in 1741. In 1753, a group of these immigrants moved to the area of Winston-Salem, N. C., where their descendants still live.[1] Over 200 persons of this group were examined. Twenty were found to have the disease. They all belong to a group of descendants of one of the original Moravian settlers of 1753. From the pedigree (Fig. 1), it may be seen that the heredity is of the simple dominant type, as men and women are equally affected.

The youngest exhibiting the corneal dystrophy was aged 7 months, the oldest 70 years. Others were distributed throughout all age groups. It is most probable that all patients, at least to some degree, had the disease at birth or in early childhood, although the condition seems to have a tendency to get more conspicuous with age. In no case could we demonstrate the development of the dystrophy in a person that previously had been known to be perfectly normal.

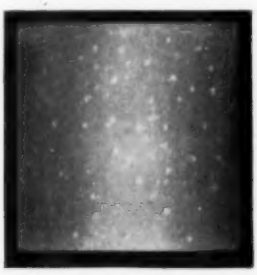


Fig. 2.—Biomicroscopic appearance of the cornea in the regredient light. The whole cornea is studded with minute droplets.

Fig. 3.—Semischematic drawing of the cornea as seen with the biomicroscope. Small whitish dots and serpiginous lines are present in the most superficial layers. The endothelium shows a normal pattern, with no signs of cornea guttata.

The most important symptom was blurred vision. Visual acuity ranged from 20/25 to 20/600, being around 20/50 in most cases. Thus, few could be termed actually blind. This may account for the fact that little attention had been attributed to the existence of the anomaly in individual cases. Other subjective symptoms were occasional itching, burning, and watering of the eyes. Many cases, however, were symptomless except for the visual disturbance.

The clinical picture of the disease was similar in all cases observed. There were, of course, some differences in the severity of the pathologic changes, but principally the same appearance was present throughout. The eye was either without or with only slight conjunctival irritation. In ordinary light the cornea appeared quite clear or showed only a minimal haze. When diffusely illuminated with the slit lamp and examined with the binocular microscope, the cornea assumed a definite haze and the anterior surface appeared studded with fine grayish punctate opacities. In most of the cases, these were spread over the entire area of the cornea. On instillation of fluorescein, the whole corneal surface showed numerous punctate stainings. In the regredient light, these opacities had the appearance of small to minute droplets (Fig. 2). With focal illumination, it could

be seen that the opacity was located in the very anterior parts of the cornea. Fine serpiginous grayish lines were also present. These were seen only in certain parts of the cornea and sometimes were arranged in whorls (Fig. 3). On examination with the narrow beam, an exact localization of the lesion could be made. It was quite clear that all the changes were located anteriorly to Bowman's membrane. The epithelial surface was slightly irregular. The small space between Bowman's membrane and the surface was irregularly opaque and showed many round white dots. It was assumed that these dots represented the cross sections of the white lines previously described as seen

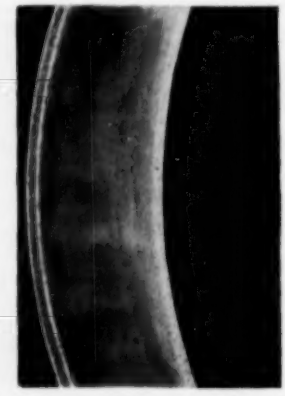
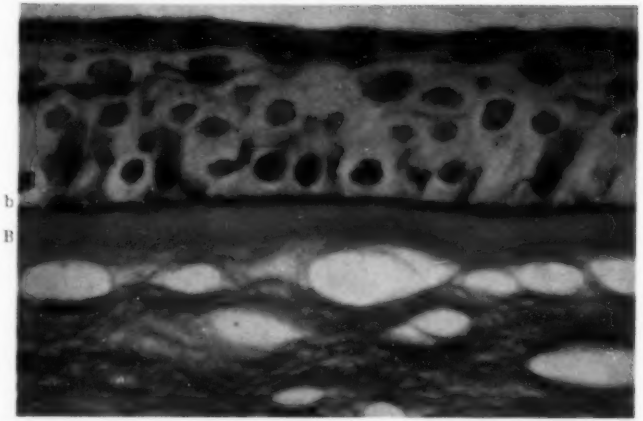


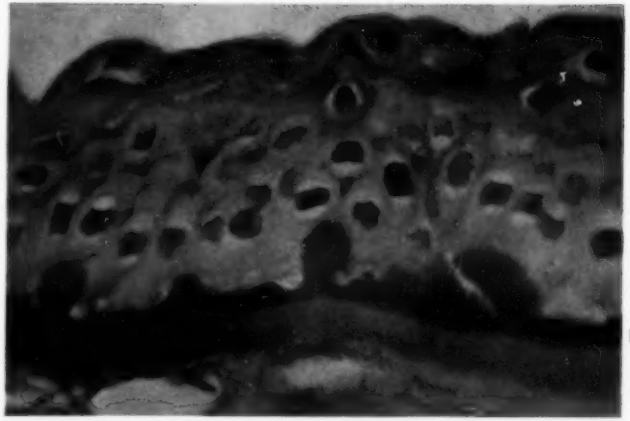
Fig. 4.—The cornea as seen in the narrow beam of the slit lamp. The small space between Bowman's membrane and the epithelial surface is diffusely opaque and shows numerous whitish dots. The corneal stroma is of normal appearance. The posterior surface of the cornea is smooth.

with focal illumination. Beneath Bowman's membrane the corneal structure was normal, showing only the differences in transparency as seen in different persons and in different age groups. Descemet's membrane was without folds, and its posterior surface was smooth (Fig. 4). There were no signs of edema of the stroma. The thickness of the cornea was uniform. In the specular or directly reflected light of the slit lamp, the epithelium had an appearance very similar to that observed in cases of corneal edema due to acute glaucoma or Fuchs's endothelial and epithelial dystrophy; that is, glittery, droplet-like elevations were spread over the entire surface of the cornea. There was no predilection for the central parts of the cornea, as is typical for early Fuchs's dystrophy. With the same technique, it could be demonstrated that the endothelium was normal, exhibiting the characteristic honeycomb pattern. No edema of the endothelial cells and no excrescences on Descemet's membrane were present (Fig. 3). The corneal sensitivity was greatly reduced in most of the cases.

In one case, in which the visual acuity was reduced to 20/400, a total lamellar corneal graft was done. The tissue removed allowed a thorough pathologic study of the diseased part of the cornea. Routine staining of sections with hematoxylin and eosin would demonstrate the lesions to some extent, but only when a modified periodic acid-Schiff stain was used could they clearly be differentiated from the other structures of the cornea.

The corneal structures back of Bowman's membrane were normal. Bowman's membrane itself stained a light purple color, only little different from the stroma, as in a normal specimen (Fig. 5). At no place was the membrane broken, allowing the pathologic process to invade the stroma. Instead of a thin basement membrane, as seen in a normal specimen, there was an amorphous layer of a dark-red-stained substance interposed between the surface of Bowman's membrane and the epithelium. Its thickness varied greatly from a slightly exaggerated basement membrane to spindle-formed obtrusions occupying as much as half the thickness of the epithelial layer. In some places, peculiarly shaped pedunculated excrescences were present (Fig. 6). Sometimes it seemed as if the basement membrane had been separated from Bowman's membrane, the space between the two being occupied by a slightly less dark-red-stained mass. In a few places a weird arrangement of alternating dark-red and light-purplish layers could be seen (Fig. 7). The epithelium itself showed marked changes. The number of layers present varied greatly. In some places there were six to seven layers of cells; in others, only two to three. The individual cells were very





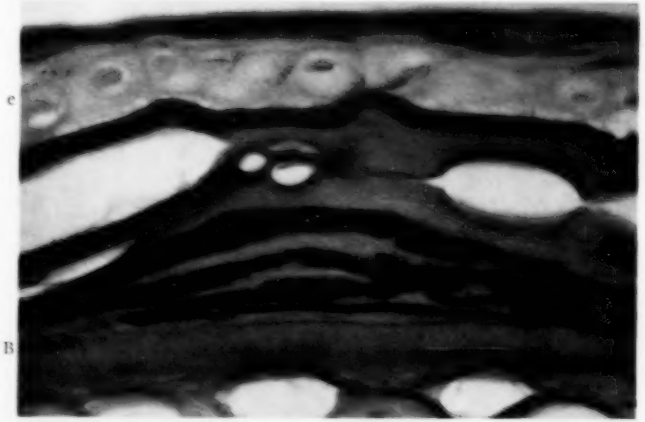


FIGURE 5. (above, left) Section through the anterior segment of a normal cornea after periodic acid–Schiff stain. The basement membrane (*b*) of the epithelium is clearly differentiated from Bowman's membrane (B). (X 743)

FIGURE 6. (above, right) Section through the anterior segment of the cornea in a case of hereditary epithelial dystrophy (same staining technique as Figure 5). The most striking features are the peculiarly shaped pedunculated excrescences (P) on the basement membrane. For further detail, see text. (X 743)

FIGURE 7. (left) Section through the anterior segment of the cornea in a case of hereditary epithelial dystrophy (same staining technique as Figures 5 and 6). A weird arrangement of alternating dark-red- and light-purple-stained layers is present between Bowman's membrane (B) and the thinned epithelial layer (e). (X 743)

irregular in shape and size. In no place could the palisade-like arrangement of the basal layer be recognized. Most of the cells were vacuolated. In some instances, several cells had merged, thus forming cystic structures. The closer to the surface the more degenerated were the cells. Sometimes, near the surface, the individual cells could no longer be identified, as they had degenerated and were transformed into an irregular stratified mass. In some instances, it could be seen that cystically enlarged cells were protruding above the surface or had burst, with extrusion of their content. Whereas the cytoplasm of the deeper layers was of a slightly purplish pink, the more superficial ones contained more dark-red material, sometimes forming clumps or granules. Some dark-red material also seemed to accumulate in the intercellular spaces, especially in the medial and superficial layers. Very few nuclei were of approximately normal appearance. Most of them showed marked changes in shape and structure. In some instances, they were completely disintegrated and reduced to darkly stained granules.

After it had become evident that, owing to a central opacity that developed in the graft, the lamellar corneal transplant had not produced the desired improvement in visual acuity, a partial penetrating graft was done on the same cornea. This made available for examination also the posterior part of the affected cornea. It could be demonstrated that Descemet's membrane and the endothelium were perfectly normal. Thus the differentiation of the disease from Fuchs's endothelial dystrophy which had been established clinically was confirmed by histologic findings.

One may speculate about the significance of these pathologic findings. The combination of periodic acid with fuchsin-sulfuronic acid (Schiff's reagent) has been widely used in the recent past to demonstrate carbohydrates in tissues by Lilly,[2] McManus,* Hotchkiss,[5] and others. In ophthalmic pathology, the method was first used by Friedenwald.[6] If the polysaccharides of tissues are treated in this way, they are identified by their ability to recolorize the Schiff stain. Several modifications and counterstains were used. Epithelial basement membranes were found by Lilly[2] to be stained red. This, of course, is true also of the corneal epithelium, as we could demonstrate in numerous routine slides from eye specimens treated with this method for general interest (Fig. 5). Recently, Teng and Katzin[7] confirmed these findings. In our case of epithelial dystrophy, it can be clearly demonstrated that the pathologic substratum is to be differentiated from Bowman's membrane by its difference in staining properties. Bowman's membrane remains intact and stains as in a normal specimen. On the other hand, this material stains not unlike Descemet's membrane. Since Descemet's membrane is thought to be secreted by the corneal endothelium, it seems logical to assume that the pathologic material in our case, which is located between the epithelium and Bowman's membrane, may be the product of the diseased epithelial cells. This theory is supported also by the fact that material of the same staining properties can be demonstrated within the epithelial cells themselves. Unfortunately, the periodic acid-Schiff stain is not specific enough to tell us exactly with what compound we are confronted. Starch, cellulose, glycogen, glycoproteins, mucins, and various other substances containing carbohydrates are stained almost alike. Efforts are being made to make the stain more specific by altering the technique and using different counterstains. But it is too early to make any statement as to the metabolic process involved in this epithelial pathology. It can be said, however, that the process is limited to the epithelium. This is an important fact for the differential diagnosis and the classification of the disease.

* References 3 and 4.

COMMENT

From the pedigree, simple dominant heredity can be demonstrated. No exclusive linkage to one sex is evident. Clinical observation and pathologic studies make it clear that the process is confined to the epithelial layers. For that reason, the disease must be differentiated from the granular and lattice type corneal dystrophies of Bücklers'[8] triad,

which are also dominant in heredity but definitely involve the deeper structures of the cornea. As far as we could see, the entity of dominant hereditary epithelial dystrophy has never been reported as such in this country. In searching the European literature, we found four reports (Pameijer,[9] 1935; Meesmann and Wilke,[10] 1939; Böck,[11] and Bürki,[12] 1946) which may have dealt with the same condition we have described. Some discrepancies, however, exist. Pameijer reported the presence of a great number of small punctate or drop-like opacities located in the very superficial layers of the cornea in a mother and her 8-year-old son, as well as in the mother's sister and in two of her three daughters, aged 11 and 9 years, respectively. Meesmann and Wilke produced a more complete report. They found 34 persons belonging to three families living in Holstein (Germany) to be affected with a corneal lesion that was composed of numerous grayish spots located in the epithelial layers of the cornea. The endothelial pattern was unaltered. In some cases, whorl-like opacities were seen and thought to be within Bowman's membrane. The condition was observed in adults and children as young as 3 years old. Dominant heredity was demonstrated. The condition was steadily progressive, with episodes of irritation occurring, especially in childhood. Some cases showed corneal scars following ulceration and secondary infections. Bürki reported 42 descendants of a presumably diseased ancestor who showed a condition similar to that reported by Meesmann and Wilke. In this group, living in Switzerland, both children and adults, men and women, were affected. Whereas Pameijer and Bürki had made no pathologic studies, Meesmann and Wilke examined scrapings of the cornea from scars which had formed after ulceration. Their findings, therefore, differ from ours in some instances. They described vacuolation of epithelial cells and, using Best's staining method, found that these contained glycogen. The authors, however, failed to demonstrate the typical exudations overlying Bowman's membrane found by us. Since our sections were made from tissue unaltered by secondary changes, our findings should be regarded as the typical pathology of this disease.

The condition most likely to be confused with the picture described here is Fuchs's endothelial and epithelial dystrophy.† There is, at first glance, a certain likeness in the appearance of the superficial layers of the cornea. Especially, the slightly protruding epithelial cells, giving the effect of "goose pimples" in the direct reflected light of the slip lamp, look quite similar. There are, however, several fundamental differences between the two. In the condition referred to here, there is no edema of the corneal stroma, which is not thickened. There are no epithelial bullae, and the endothelium shows a normal pattern, excrescences on Descemet's membrane being wholly absent.

Another type of corneal dystrophy showing some similarity to the cases we are reporting is the so-called "whorl-type" dystrophy or "cornea verticillata," reported by Fleischer[13] (1910), Lutz[14] (1930), Vogels[15] (1931), and Gruber[16] (1946). All these authors describe a condition that is characterized by the presence of numerous grayish lines arranged in a whorl-like manner and spread over the whole cornea. These may, at first glance, have some similarity to the white serpiginous lines described in our cases. The epithelium, however, is smooth, and the lines are located either within Bowman's membrane or in various parts of the stroma. Although there seems to be dominant heredity, as in our cases, there is sufficient difference in the two forms to permit their classification as different entities. Also, the two forms do not occur concurrently in any group of persons.

One other condition to be considered for differential diagnosis is the type of dystrophy reported by Bücklers,[17] 1949, which is characterized by a glassy appearance of the cornea. Bowman's membrane appears to be broken up and split, and grayish figures are present in Bowman's membrane. The deeper stroma is not markedly affected. Bücklers

† For detailed reference on Fuchs's dystrophy, see Stocker.[18]

himself rightly differentiates his cases from all other dystrophies, including the one reported by Meesmann and Wilke.

Finally, as to the possibility of influencing the course of this corneal dystrophy by therapeutic measures, in most instances no treatment is necessary as long as the visual acuity is not drastically reduced. In the severer cases, clearing agents, such as glycerin, or cortisone and hydrocortisone, were tried, but with no avail. The only logical and effective treatment to be applied in severe cases appears to be corneal transplantation. Since the lesion is definitely confined to the most superficial parts of the cornea, anteriorly to Bowman's membrane, lamellar corneal grafting may be indicated. We performed this operation in one case but did not obtain any substantial improvement of vision, evidently because a centrally located corneal opacity had developed. A subsequent partial perforating graft, 7 mm. in diameter, improved vision from 20/400 to 20/60.

SUMMARY

A rare familial epithelial corneal dystrophy is described which was found to be present in 20 persons comprising four generations, all descendants of a Moravian settler who came to the area of Winston-Salem, N. C., in 1753. Simple dominant heredity was demonstrated.

The purely epithelial character of the disease was confirmed by biomicroscopy and pathologic studies. The lesions, located between Bowman's membrane and the epithelium, were clearly differentiated from Bowman's membrane by the periodic acid-Schiff stain.

The only treatment advised for the severer cases is corneal transplantation, which was successfully performed in one case.

Biomicroscopic drawings are by Mr. R. O. Beach, Department of Medical Illustration, and the photomicrographs, by Mr. Carl M. Bishop, Department of Pathology, of Duke University School of Medicine.

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Effects of Illumination and Sleep upon Amplitude of Electro-Oculogram

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The electro-oculogram (EOG) may be defined as a record of eye movement based on the detection of the D. C. potential of the eye and subsequent electronic magnification. Depending upon whether a D. C. or a capacitance-coupled amplifier is employed, the amplitude of the EOG will be subject to different interpretation. With a D. C. amplifier, for instance, the amplitude of the EOG will bear a relationship to the angle of ocular rotation. However, regardless of the type of instrument used, it is evident that the resultant EOG must also be a function of the magnitude of the resting potential of the eye. Experimental procedures which alter the eye potential can be expected to affect the EOG accordingly. Although several papers * relate their results to the D. C. potential of the eye, the relationship is indirect, inasmuch as EOG's rather than eye potentials were recorded.

Significant changes of the D. C. potential of the rabbit eye have been wrought by illumination and metabolic inhibtors.[4] In man, the EOG has been modified by acapnia induced through hyperventilation.[1] However, reports on the effect of dark and light adaptation of the eye upon the EOG in man are not entirely clear. Miles [2] reported that the eye potential (EOG) was significantly higher when recorded from the closed eye. Later Miles [3] indicated that at the end of 5 and 10 minutes at two levels of illumination, the eye potential (EOG) was definitely lower when recorded from the dark-adapted eye. Callahan and Redlich [5] reported that blink waves (EOG of the eye movement during blinking) were identical when recorded in daylight and in complete darkness.

The present report deals with a series of tests conducted on five men to determine the effects of retinal adaptation and of sleep upon the amplitude of the electro-oculogram.

METHOD

Pick-up electrodes were applied to the inner and external canthi of the right eye and the potentials led to a Grass electroencephalograph set at the longest (EKG) time constant. The subjects were instructed to fixate alternately upon two dimly lit ophthalmoscope bulbs placed about 15 ft. away so that the eye rotated horizontally over an angle of 23 degrees. From 4 to 10 swings were executed in each direction. This was performed after light adaptation of 5 to 15 minutes' duration in a moderately illuminated room and after dark adaptation of 5 to 17 minutes in a room completely dark except for the two fixation lights, which were slightly above the dark-adapted visual threshold.

Records were taken after successive alternate periods of dark and of light adaptation prior to going to sleep. Four subjects were retested immediately upon awakening the following morning. The room was maintained completely dark throughout the period of sleep so that the initial test in the morning could be conducted in the absence of prior light conditioning.

The mean EOG potentials for nasalward and temporalward movement at the end of the first period of dark adaptation prior to sleep were arbitrarily set at 100 units respectively. Then the EOG values for the succeeding tests were converted to units based on these standards. Since the EOG's for nasalward and temporalward movements presented essentially identical results, the values in

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From the Physiology Department of the University of Chicago. This study was carried out under a U. S. Public Health Service Fellowship.

* References 1 through 3.

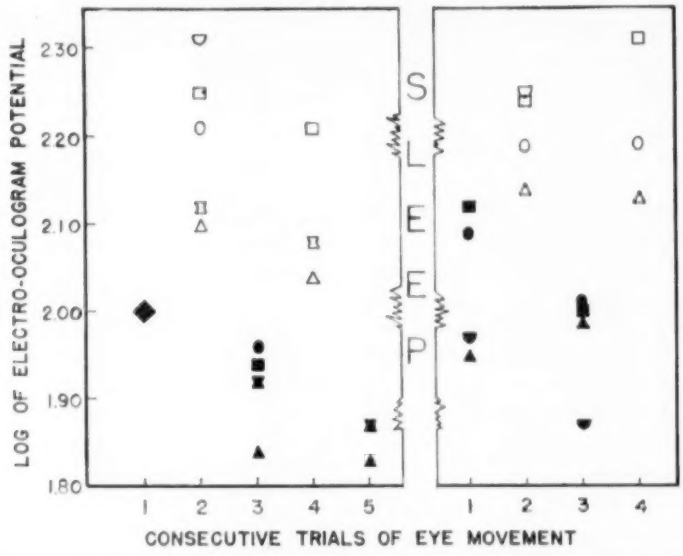

Fig. 1.—Changes in amplitude of the electro-oculogram after successive periods of dark and light adaptation both before and after sleep. The symbol (diamond) denoting the EOG potential at the end of the first period of dark adaptation before sleep was designated as 100 units for each subject. Each of the remaining symbols represents a specific subject; the symbols when open indicate the EOG potential at the end of a period of light adaptation, and when closed, the potential at the end of a dark adaptation period.

this report are based on the averages of the arbitrary units derived for both the nasalward and the temporalward movements. Thus "80" would represent an average reduction of the EOG potential by 20% with reference to the value obtained for that subject in the first test before going to sleep. The absolute values of the EOG potentials for the individual subjects at the start of the experiment were 182, 183, 203, 218, and 247μv respectively.

RESULTS

For graphic purposes some of the results (Fig. 1) are expressed as logarithms of the arbitrary EOG units which were described above. At the end of approximately 10 minutes of light adaptation, the EOG amplitude was significantly ($p < 0.01$) higher than at the end of a comparable period of dark adaptation. This increase ranged from 11% to 208%, depending upon the individual and other circumstances. Superficial examination of the data suggested that succeeding periods of dark adaptation resulted in lower and lower EOG amplitudes until a plateau was reached. A statistical analysis supported that observation. The mean EOG amplitude of the second period of dark adaptation before sleep was lower ($p < 0.05$) than the mean value for the first period of dark adaptation before sleep. Although the second period of dark adaptation after sleep yielded a similarly lower mean EOG value than the preceding period of dark adaptation, this difference was not statistically significant. However, by combining the data from the night and morning tests, and securing mean values for the subjects, the EOG amplitude of the second dark adaptation period was found to be significantly ($p < 0.001$) lower than it was at the end of the previous dark adaptation period. The means for the EOG's at the end of the second and third periods of dark adaptation before sleep were practically the same.

One might have expected that an extremely long period of dark adaptation, such as ensued with a night's sleep, would either have further lowered or have not changed the EOG amplitude relative to the second or third dark adaptation period just prior to sleep. The contrary was suggested by the data. Immediately

upon awakening in the morning, the mean EOG amplitudes were from 29% to 51% higher ($p < 0.02$) than at the end of the second dark adaptation period of the night before.

The EOG amplitude at the end of succeeding periods of light adaptation showed a downward trend before sleep, but this was not statistically significant.

Samples of EOG amplitude taken at approximately minute intervals during light and dark adaptation confirmed in general the observation that an increasing level of dark adaptation resulted in a decrease of EOG amplitude, whereas an increasing level of light

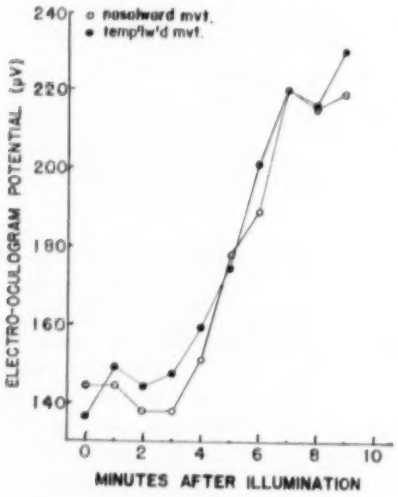

Fig. 2.—Electro-oculogram amplitude during the course of light adaptation of the eye. The tests were conducted immediately after a 10-minute period of dark adaptation.

adaptation caused an increase of EOG amplitude. However, the changes of EOG amplitude were not necessarily linear. A typical EOG curve during light adaptation (Fig. 2) indicated that the EOG amplitude did not change radically until the illumination had been on for three or four minutes; in fact, there was an initial decrease in amplitude at the end of the first minute in two cases. On the other hand, the data suggested that the EOG amplitude decreased steadily from one minute after illumination was turned off until a plateau was reached, about five or more minutes later.

COMMENT

Although the EOG potential was elevated by illumination, the change did not appear to be linear with time. Retinal light adaptation is extremely rapid and generally completed in one to two minutes (Adler [6]). Yet, the EOG amplitude at the end of one minute's light adaptation has hardly begun to rise or may, indeed, have fallen. This failure of the EOG to parallel sensory adaptation is in accord with Noell's postulation [4] that the major component of the D. C. potential of the eye has its origin in the retina-choroid barrier rather than in the activity of the sensory cells.

In view of the few subjects involved in these tests, the apparent increase in EOG amplitude after a night's sleep as compared with the amplitude at the end of a dark adaptation period immediately prior to retiring must be accepted for generalization with caution. Nevertheless, in retrospect such a change would not be surprising. The increased EOG amplitude could possibly have resulted from a metabolic change directly affecting the magnitude of the D. C. potential of the eye. On the other hand, since a capacitance-coupled amplifier (Grass) was employed, the amplitude of the EOG could have been affected by changes in velocity of eye movement. An increased velocity in the morning could have accounted for the observed EOG change. That the latter explanation is distinctly possible is supported by evidence [7] that there is a diurnal variation of the velocity of eye movement.

The D. C. potential of the eye has been styled as a "steady" potential, in contrast to a phasic potential such as exhibited by the electroretinogram. However, the term "steady" may be misleading. Fenn and Hursh [8] have shown that the EOG potential has a relatively small variance in day-to-day measurements on the same subject. Yet this variance can assume tremendous importance in the absence of large samples. For example, in the above-mentioned paper,[8] seven measurements on dif-

ferent days for the same subject ranged from 0.16 to 0.25 mv., an extreme variation of about 50%.

Certain conclusions which have depended on accurate measurement of EOG amplitude and consequently on the "steadiness" of the d. c. potential of the eye may require reevaluation. Bergman, Nathanson, and Bender [9] concluded on the basis of EOG amplitude that sodium amobarbital reduced the velocity of gaze movements and of blinking. Yet it is possible that the reduction of EOG amplitude was due to an effect of the drug upon the d. c. potential of the eye rather than upon oculomotor performance.

In investigations where ocular motility was judged quantitatively from the EOG while the subject's eyes were shut in order to differentiate certain pathological conditions [10] or to measure the intensity of nystagmus,[11] the question arises as to whether uncontrolled duration of eye closure in itself may have altered the amplitude of the EOG through a dark adaptation effect. Furthermore, if a second trial were executed with the eyes closed, an additional change in the EOG may have resulted.

Finally, there is the decision reached by Lion and Brockhurst [12] that in a severe stress situation involving extremely rapid movement of the eyes over a 90-degree arc, some of the subjects eventually failed to move their eyes over the complete distance. This judgment, too, was based on the amplitude of the EOG. They explained this phenomenon as due to the subjects' becoming satisfied with an extrafoveal image. However, it is questionable whether normal eyes can fixate extrafoveally. According to Adler,[13] the fixation reflex is extremely potent and, once the image is within a few degrees of the macula, will cause the eye to move reflexly so that the fovea will receive it. From a sample record illustrated in Lion and Brockhurst's paper,[12] the amplitude of the EOG (d. c. amplifier recording) was reduced about 14% at the end of 5 minutes of stress and about 27% at the end of 10 minutes. If this is to be interpreted as due solely to the eyes' traversal over a shorter arc, then, according to the relationship [8] established between the EOG amplitude and the degrees of ocular rotation, the eyes, after 10 minutes, moved circa 60 degrees instead of the initial 90 degrees. Consequently, the image would fall about 15 degrees peripheral to the fovea and the visual acuity would be reduced [6] to less than 20% of the acuity for the fovea. Extrafoveal fixation, if at all possible, would have had to be performed at a relatively low level of visual acuity, and an unverified habit formation would have had to intervene. A plausible alternative explanation for the observed reduction of EOG amplitude might be in the possibility that the d. c. potential of the eye was changed by or during the experimental procedure.

SUMMARY

Dark and light adaptation of the eye significantly alter the amplitude of the electro-oculogram, but the changes do not appear to parallel the temporal course of retinal adaptation. The EOG amplitude of the dark-adapted eye decreases with subsequent testing under the conditions of alternating light and dark adaptation trials.

Sleep may increase the EOG amplitude above a presleep minimum level, but it is not known whether this effect is due entirely to a diurnal increase of eye movement velocity or to an increase of the d. c. potential of the eye.

The interpretation of the results of other investigations are discussed in view of the possible variability of the electro-oculogram as a result of changes in the magnitude of the resting potential of the eye which occurred unintentionally.

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News and Comment

EDITED BY DR. W. L. BENEDICT

GENERAL NEWS

Postgraduate Course in Ophthalmology, Brazilian Society of Ophthalmology.—The First Postgraduate Course on Ophthalmology, sponsored by the Brazilian Society of Ophthalmology, took place in Rio de Janeiro, Jan. 10 to Feb. 5, 1955. Dr. Almiro Azeredo was the director, and the teachers were Professors Abreu Fialho, Almiro Azeredo, Barbosa Luz, Evaldo Campos, Jonas Arruda, Joviano Rezende, Luis Novias, Luiz Eurico Ferreira, N. Moura Brazil, Paulo Pimental, Rafael Benchimol, R. Fernandes, R. Rolin, and Werter Duque Estrada.

The following courses were offered:

First week: Anatomy, histology, and physiology related to the eye, including laboratory studies
Second week: Eye examination and pathology
Third week: Eye examination and pathology
Fourth week: Eye surgery, including the training of surgery in the eyes of animals

Attendance was limited to 16; tuition was 1000 cruzeiros.

The Second Postgraduate Course will be held from Feb. 28 to March 26, 1956.

American Academy of Ophthalmology and Otolaryngology Home-Study Courses.—The 1955-1956 Home Study Courses in the basic sciences related to ophthalmology and otolaryngology, offered as a part of the educational program of the American Academy of Ophthalmology and Otolaryngology, will begin on Sept. 1 and continue for a period of 10 months. Detailed information and application forms can be secured from Dr. William L. Benedict, the executive secretary-treasurer of the Academy, 100 First Avenue Building, Rochester, Minn. Registrations should be completed before Aug. 15.

PERSONAL NEWS

Gold Medal of Centro de Estudos de Ophthalmologia Awarded.—At a special meeting held in the main auditorium of the Paulista Medical Association on Feb. 12, 1955, Dr. Moacyr E. Alvaro was awarded the Gold Medal of the Centro de Estudos de Ophthalmologia for his contribution to the progress of Brazilian ophthalmology. After receiving the medal, Dr. Alvaro delivered the Centro de Estudos de Ophthalmologia Lecture on "Recent Trends in Organized Ophthalmology."

Experimental Leptospiral Uveitis in Rabbits

RUDOLF H. WITMER, M.D., New York

The results of previous clinical and experimental work with horses suffering from periodic ophthalmia strongly suggest that this uveitis is due to Leptospira pomona and L. grippotyphosa infection,* although the leptospiral nature of periodic ophthalmia could be proved by culture in only a few cases. Hertel [3] (1917) produced uveitis in rabbits by injecting suspensions of Leptospira icterohaemorrhagiae into the vitreous and into the anterior chamber. Gsell [4] reported leptospiral infections in humans, with an occasional involvement of the uvea. These authors did not conduct any serological studies on aqueous humor. Recent experimental data on horses suffering from periodic ophthalmia provided strong evidence of local antibody formation. Similar results were obtained in experimental tuberculous uveitis in rabbits.[6] Since it has been reported [5] that rabbits, generally resistant to systemic leptospiral infection, have high antibody titers, this test lesion appeared to be especially suited to the investigation of local antibody formation in ocular infections.

The experiments were carried out along three lines.

In a first group the primary intraocular infection with leptospires was studied. In a second experiment the problem of reinfection was investigated. In a third series a local irritant (caterpillar hair) was used as an "adjuvant," combined with primary leptospiral infection. Data were obtained on the clinical course of the infection and on the quantitative relationship between Leptospira-agglutinating antibodies in serum and those in aqueous humor. The gamma globulin content was determined by means of paper electrophoresis, and the ratio of titers to gamma globulin content was recorded. Histological examinations at different stages of the infection supplemented the immunological studies.

MATERIAL AND METHODS

The sera of all rabbits were tested prior to the infection and were found to contain no Leptospira-agglutinating antibodies. One-tenth milliliter of a 1:10 dilution of a 10-day-old culture of living leptospires in Schuffner's liquid medium, that is, approximately 2,000,000 organisms,† was injected, with the animal under ether anesthesia, into the vitreous body of the right eye only. The injection into the vitreous was chosen with the idea that by such procedure the organisms would remain in the eye longer than by injection into the anterior chamber, and that a systemic infection would thus be delayed. The left eyes were not inoculated. In control rabbits 0.1 ml. of plain Schuffner's medium was introduced into the vitreous. To minimize interference with the natural course of the inflammation, aqueous of the infected eyes was aspirated at intervals of 10 days. A tuberculin syringe with a 24- or 26-gauge needle was used to withdraw these samples of aqueous humor from the anterior chamber. Blood samples were taken by bleeding the marginal ear vein.

Caterpillar hair was cut with scissors from living caterpillar Malacosoma americana and used as a local irritant. The hair was suspended in saline, washed once with saline, and then autoclaved for 20 minutes at 15 lb. pressure. The heavy suspension of hair in saline was then stored in the ice box.

Extracts of uveal tissue were made by bisecting the freshly enucleated eyes with a microtome knife. Lens, vitreous body, and retina were removed, and the uveal tissue (iris, ciliary body, and choroid) was dissected with scissors, placed in a centrifuge tube, frozen and thawed three times in an alcohol-solid

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This investigation was aided by grants of the Knapp Memorial Foundation, the Snyder Ophthalmic Foundation, and the Swiss Academy of Medicine.

* References 1 and 2.

† The leptospires were counted according to the technique of H. J. Magnuson.[7]

carbon dioxide (dry ice) mixture, and ground for 15 minutes with small glass beads. The tissues were then centrifuged at 3000 rpm for 30 minutes and the 0.02 to 0.03 ml. of clear supernatant tissue fluid was pipetted off for further processing.

Antibodies in serum and aqueous humor were determined by direct agglutination-lysis reaction [5] with a five-day-old culture of the same strain of L. pomona in Schüffner's [8] liquid medium and observation with dark-field microscopy. The titers were recorded as reciprocals of the highest dilution showing agglutination.

Paper electrophoresis, according to the method of Grassmann, Hannig, and Knedel,[9] was performed on serum and aqueous humor. This procedure gives reasonably accurate results in serum with an average error of not more than 2% to 3%. However, the examination of aqueous humor renders reliable results only when the protein content exceeds 0.5 gm. per 100 cc. In samples with a lower protein content identification of the different fractions is doubtful (average error 5% to 8%). The protein content of the serum was determined by the micro-Kjeldahl method, and the protein content of the aqueous humor was calculated from the electrophorograms, as described previously.[10] Since the determination of the agglutination end-titers in aqueous humor and serum did not provide sufficient information and most antibodies are gamma globulins, the titers are related to the gamma globulin content of the two fluids. In order to simplify the expression of the results, the reciprocals of the highest dilution showing agglutination are related to 1 gm. of gamma globulin, as measured by paper electrophoresis. This ratio is called "antibody activity."

The rabbits were examined twice a week with ophthalmoscope and slit lamp. Within the course of the experiment 14 animals were killed and the eyes enucleated for histological studies. Kidney

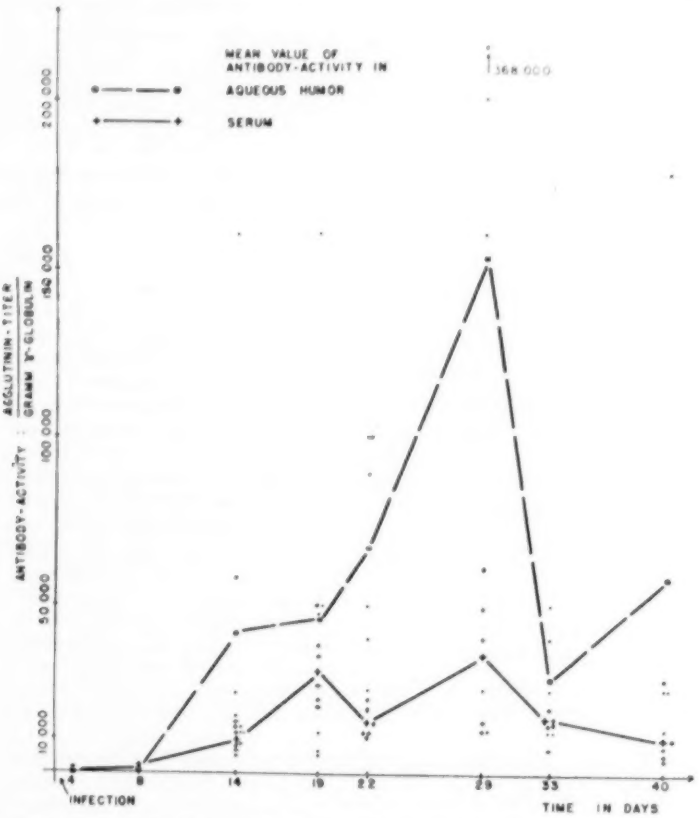


Fig. 1.—Course of antibody activity of serum and aqueous humor after primary intraocular infection with Leptospira pomona.

samples and aqueous humor samples were taken at the time the animal was killed and cultured in Fletcher's [8] semisolid, Schüffner's liquid, or Korthoff's [5] liquid medium. The aqueous humor was also subjected to dark-field studies.

RESULTS

1. *Primary Infection.*—In the first group (18 normal chinchilla rabbits of both sexes, average weight 4 to 5 lb. [1.8 to 2.3 kg.]) the intravitreous injection of approximately 2,000,000 living leptospires produced an acute iritis, which appeared on the third and fourth days and lasted about one week. After that time the inflammation slowly decreased and cleared completely in four to six weeks. A chronic uveitis, lasting more than six weeks, developed in three rabbits. There was marked variation in the individual reactions of the experimental animals. The uninoculated eyes of all rabbits remained normal. The eyes of the control animals, which had been injected with plain Schüffner's medium, showed a mild irritation the first day, but no uveitic lesions.

Dark-field examinations and cultures of fresh samples of aqueous humor revealed living and motile leptospires to be present in the anterior chamber as long as eight days

TABLE 1.—*Antibody Activity* [*] *of Primary Aqueous Humor in Normal Eyes in Primary Infected Rabbits*

Rabbit No.	Days After Infection	Observed Titers		Titer/Gm. Gamma Globulin		Ratio [†]
		Serum	Aqueous	Serum	Aqueous	
2	30	40,000	200	49,000	33,000	0.67
3	30	15,000	25	15,000	4,100	0.27
4	30	40,000	100	61,000	16,700	0.27
5	30	10,000	25	12,500	4,100	0.33
6	30	40,000	25	40,000	4,100	0.10
12	33	20,000	25	16,400	4,100	0.25
13	33	30,000	50	19,400	8,300	0.42
14	33	15,000	25	10,000	4,100	0.41
15	33	20,000	25	14,000	4,100	0.29
16	33	20,000	25	15,000	4,100	0.27
Average				25,290	8,670	0.32

[*] Calculation based on a protein content of 0.08 gm. per 100 cc. and a gamma globulin content of 20%, i. e., 0.016 gm. per 100 cc.

[†] Ratio: $\frac{\text{antibody activity of aqueous humor}}{\text{antibody activity of serum}}$

TABLE 2.—*Secondary Aqueous Humor in Normal Eyes in Primary Infected Rabbits*

Rabbit No.	Days After Infection	Observed Titers		Titer/Gm. Gamma Globulin		Ratio [*]	Gamma Globulin Content, Gm./100 Cc.	
		Serum	Aqueous	Serum	Aqueous		Serum	Aqueous
2	30	40,000	8,000	49,000	15,000	0.31	0.81	0.52
	48	40,000	8,000	70,000	20,000	0.28	0.57	0.40
3	30	15,000	2,000	15,000	4,350	0.29	1.04	0.46
	48	8,000	1,000	8,900	1,200	0.13	0.90	0.78
4	30	40,000	8,000	61,000	30,000	0.50	0.65	0.26
	48	20,000	4,000	17,000	7,400	0.43	1.12	0.54
5	30	10,000	2,000	12,500	3,570	0.28	0.80	0.56
6	30	40,000	8,000	40,000	16,540	0.41	0.98	0.48
	48	16,000	1,000	20,000	2,400	0.12	0.77	0.41
12	33	20,000	4,000	16,400	8,000	0.49	1.22	0.52
	39	8,000	500	6,600	1,470	0.22	1.20	0.34
14	33	15,000	2,000	10,000	4,700	0.47	1.47	0.42
	39	4,000	1,000	5,300	2,000	0.38	0.69	0.55
15	33	20,000	2,000	14,000	2,400	0.17	1.39	0.83
	39	8,000	2,000	6,200	2,600	0.42	1.28	0.75
16	33	20,000	2,000	15,000	2,600	0.17	1.31	0.76
	39	8,000	2,000	8,000	2,350	0.29	1.06	0.86
Average				22,100	7,460	0.31	1.01	0.55

[*] Ratio: $\frac{\text{antibody activity of aqueous humor}}{\text{antibody activity of serum}}$

TABLE 3.—*High Protein Content Primary Aqueous Humor in Primary Infected Eyes*

Rabbit No.	Days After Infection	Observed Titers		Titer/Gm. Gamma Globulin			Gamma Globulin Content, Gm./100 Cc.	
		Serum	Aqueous	Serum	Aqueous	Ratio *	Serum	Aqueous
21	6	800	200	500	600	1.20	1.61	0.32
9	12	10,000	4,000	7,220	12,000	1.66	1.38	0.53
12	12	10,000	2,000	4,940	11,950	2.42	2.06	0.16
13	12	5,000	1,000	6,450	16,400	2.54	0.77	0.06
14	12	10,000	200	13,500	13,400	0.99	0.74	0.17
15	12	10,000	4,000	11,350	24,500	2.16	0.88	0.16
16	12	10,000	2,000	13,070	58,800	4.50	0.76	0.03
	20	15,000	400	15,000	40,000	2.67	0.98	0.01
17	15	16,000	1,000	19,000	20,000	1.05	0.82	0.05
23	15	4,000	2,000	4,000	4,000	1.00	0.99	0.46
2	18	30,000	200	26,600	5,280	0.19	1.12	0.08
	28	40,000	64,000	49,000	368,000	7.50	0.81	0.17
	39	20,000	32,000	28,000	178,000	6.40	0.71	0.18
	47	40,000	32,000	70,000	112,000	1.60	0.57	0.28
Average				21,800	66,500	2.56	1.07	0.18

* Ratio: $\frac{\text{antibody activity of aqueous humor}}{\text{antibody activity of serum}}$

TABLE 4.—*Low Protein Content of Primary Aqueous Humor in Primary Infected Eyes*

Rabbit No.	Days After Infection	Observed Titers		Titer/Gm. Gamma Globulin			Gamma Globulin Content, Gm./100 Cc.	
		Serum	Aqueous	Serum	Aqueous	Ratio *	Serum	Aqueous
18	16	2,000	50	2,000	6,200	3.10	1.03	0.008
19	16	16,000	100	11,000	5,000	0.45	1.44	0.020
20	16	8,000	1,000	6,700	15,000	2.24	1.19	0.065
21	16	2,000	50	1,400	600	0.42	1.40	0.008
3	18	40,000	100	35,000	6,400	0.18	1.14	0.015
4	18	30,000	1,600	38,000	160,000	4.20	0.79	0.010
5	18	15,000	200	22,000	12,000	0.54	0.68	0.016
6	18	10,000	800	20,000	47,000	2.35	0.50	0.017
8	18	40,000	200	40,000	50,000	1.25	1.05	0.004
10	12	10,000	3,200	8,200	160,000	19.50	1.22	0.020
	20	30,000	300	19,000	50,000	2.63	1.58	0.004
12	20	15,000	800	11,000	89,000	8.10	1.36	0.009
13	20	15,000	400	11,200	100,000	8.90	1.34	0.004
14	20	40,000	100	22,700	25,000	1.10	1.76	0.004
15	20	15,000	400	11,700	100,000	9.02	1.27	0.004
3	28	15,000	100	15,000	12,500	0.83	1.04	0.008
4	28	40,000	1,600	61,600	160,000	2.60	0.65	0.010
5	28	10,000	400	12,500	25,000	2.00	0.80	0.016
6	28	40,000	1,600	40,000	200,000	5.00	0.98	0.008
12	31	20,000	400	16,400	40,000	2.50	1.22	0.010
13	31	30,000	200	19,400	12,500	0.64	1.55	0.008
14	31	15,000	100	10,000	12,500	1.25	1.47	0.008
15	31	20,000	400	14,000	50,000	3.57	1.39	0.008
16	31	20,000	200	15,000	25,000	1.66	1.31	0.008
12	37	10,000	200	10,000	25,000	2.50	0.98	0.008
14	37	10,000	100	7,000	12,500	1.24	1.42	0.008
15	37	10,000	200	5,000	25,000	5.0	2.16	0.008
16	37	5,000	400	4,200	50,000	11.90	1.17	0.008
Average				17,500	52,700	3.76	1.21	0.015

* Ratio: $\frac{\text{antibody activity of aqueous humor}}{\text{antibody activity of serum}}$

after the infection. The organisms could either be seen under the microscope or be cultured in the above-mentioned media in 14 of the 18 rabbits which were examined during the first week after the infection. In rabbits examined 12 days after the infection or later the results of the tests were negative.

Leptospira-agglutinating antibodies were first detected in the serum eight days after the infection and in the aqueous humor 14 days after the infection. The titers of serum and aqueous humor rose sharply from the end of the first week up to the fourth week, then decreased during the following weeks. Figure 1 represents the mean values of the antibody activity in the aqueous humor as compared with that of the serum of five to eight animals with primary infection. Antibody activity of the aqueous humor exceeded by far the activity of the serum 14 days after the infection and reached a peak value on the 29th day. During the period in which the serum titer was high, that is, the third to the fifth week, small amounts of antibodies were present in the primary aqueous humor of the noninfected eye (Table 1). The protein content in normal aqueous humor of rabbits is known to range between 0.02 and 0.04 gm. per 100 cc. and is not detectable by means of paper electrophoresis. The calculations in Table 1 were based, therefore, on an assumed protein content of 0.03 gm. per 100 cc. and a gamma globulin content of 0.006 gm. per 100 cc. (i. e., 20% of the total protein). The results shown in Table 1 indicate that the antibody activity of the primary aqueous humor is lower than that of the serum. The secondary aqueous humor in the noninfected eyes‡ (Table 2) revealed much higher titers. The average protein content was more than half of the serum value and the gamma globulin content accurately measurable. The antibody activity of the serum averaged 22,100, and that of the secondary aqueous humor, 7460. The antibody activity

‡ The secondary aqueous humor of the normal eyes was collected 30 minutes after the first puncture, at which time the anterior chamber had usually regained its normal depth and 0.15 ml. of liquid could be withdrawn.

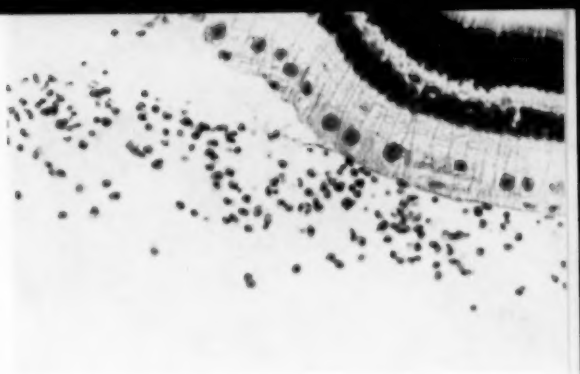
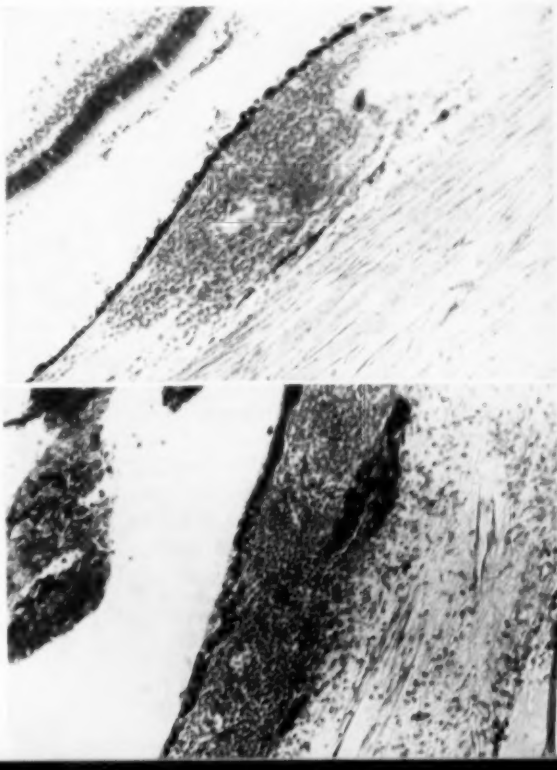


Fig. 2 (Rabbit 11).—Five days after leptospiral intraocular infection. Agglutination titers negative in serum and aqueous humor; infiltration of vitreous body by many polymorphonuclear neutrophiles, macrophages, and lymphocytes.

ratio of primary and secondary aqueous humor versus serum was the same, that is, 0.3. This shows that less than a third of the total amount of serum antibodies passes the blood-aqueous barrier of normal eyes, even when its permeability has been altered by previous paracentesis. The increase in the gamma globulin content of the secondary aqueous (50% of the serum level) is therefore at variance with the increase of anti-

Fig. 3 (Rabbit 9).—Twelve days after primary leptospiral infection. Antibody activity of serum, 7220; of aqueous humor, 12,000. Dense infiltration of deep layers of iris stroma and anterior part of the choroid by many plasma cells and lymphocytes.



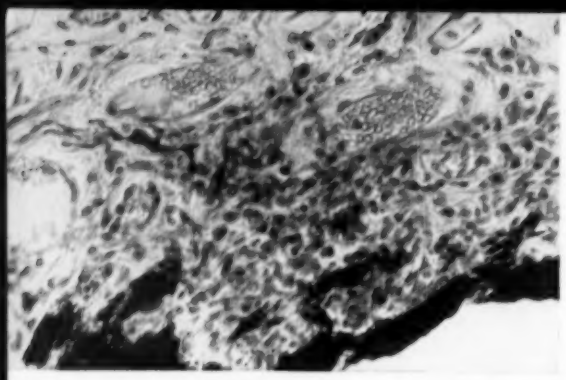


Fig. 4 (Rabbit 13).—Thirty-one days after primary leptospiral intraocular infection. Antibody activity of serum, 19,400; of aqueous humor 12,500. Spot of plasma cell infiltration in iris stroma.

results were divided into two groups. The first group, of 10 eyes (14 determinations), showed at the time of puncture a high protein content of the aqueous humor (Table 3); the antibody activity ratio of aqueous humor to serum was in most determinations (11 of 14) greater than 1, with a mean value of 2.56. In the second group, of 15 eyes (28 determinations), in a stage of slight inflammation, the average ratio of the two antibody activities was calculated as 3.76 (Table 4).

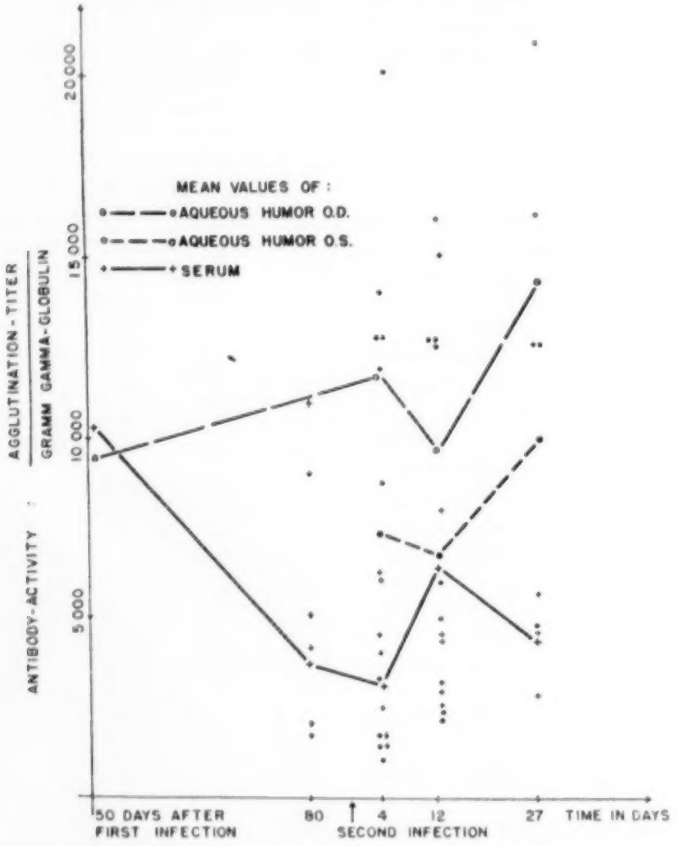


Fig. 5.—Course of antibody activity of serum and aqueous humor after intraocular reinfection with Leptospira pomona.

bodies in this fluid. The results of the experiments on primary and secondary aqueous humor from normal eyes served as a basis for the evaluation of the data obtained from infected eyes. For purposes of analysis the

The difference between the two ratios might indicate a higher activity of the aqueous humor in the second group. However, the gamma globulin content of these samples was too low to allow accurate evaluation.

For histological study, 14 rabbits were killed at periods varying from 5 to 101 days after inoculation. Early in the infection, from the 5th to the 10th day, a cellular reaction, consisting of many polymorphonuclear neutrophiles, macrophages, and a small number of lymphocytes, was seen mainly in the vitreous. These cells apparently migrated from dilated vessels of the optic disc and neighboring superficial layers of the retina (Fig. 2). Iris, ciliary body, and choroid showed hyperemia with swelling of the endothelial cells of the blood vessels, but no cellular infiltration. By the 13th to the 17th day after the infection a dense infiltration by lymphocytes and plasma cells was apparent in the deep layers of the iris stroma and the anterior part of the choroid (Fig. 3); the ciliary processes showed mainly extensive edema and hyperemia. The polymorphonuclear neutrophiles in the vitreous had disappeared and were replaced by plasma cells. By 30 days and later there were foci of plasma cell infiltration in the iris and sometimes the ciliary processes (Fig. 4). Later these cells slowly disappeared, and after three months the eyes seemed normal except for some edema of the iris and ciliary processes and a few scattered plasma cells deep in the iris stroma.

2. *Reinfection.*—In this experiment, nine animals, survivors of the first unilateral inoculation, were reinfected in both eyes after two and three months. The selected dose was approximately 10 times as high (undiluted 10-day-old culture) and the number of injected organisms was roughly 20,000,000. Examinations of aqueous humor and sera were made 4, 12, and 27 days after the second infection. In addition, the same dose was introduced into the right eye of one normal rabbit, which served as a control.

All rabbits showed an acute fibrinous reaction in both eyes during the first four days. There was not a striking difference between the right (previously infected) and the left (previously normal) eye. After eight days the inflammation of all reinfected eyes had decreased considerably, while the left eye exhibited minimal signs of inflammation. After three weeks the left eyes were almost normal,

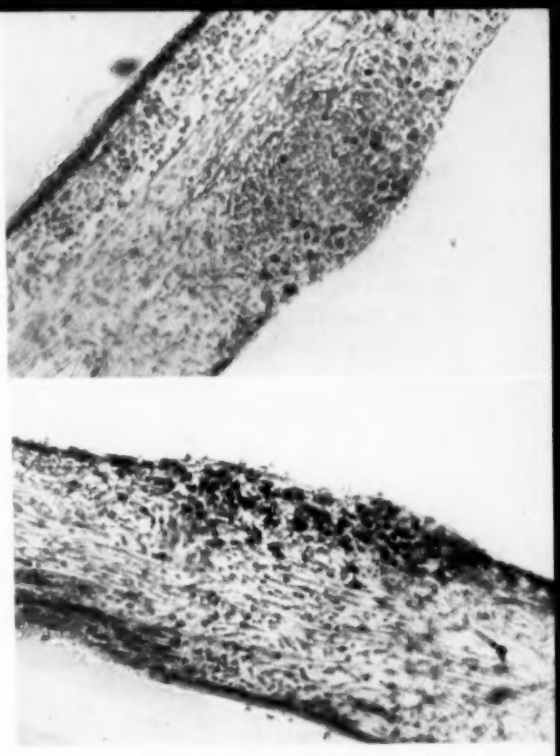


Fig. 6 (Rabbit 19).—Thirty-two days after leptospiral intraocular infection. Antibody activity of serum, 5600; of aqueous humor, 21,000. Nodules in iris stroma with a few epithelioid cells, many plasma cells, and some pigmentophores.

while some of the right eyes still presented the picture of a mild chronic uveitis. A very violent reaction, with fibrinous iritis, followed by a chronic uveitis lasting as long as 12 weeks, occurred in the control rabbit. By the fourth day after the second infection, observation with the dark-field microscope revealed leptospires in only two samples of aqueous humor, one from the right and one from the left eye, out of the entire group of reinfected animals, and only one culture was positive. At the same time, many motile leptospires were observed in the aqueous humor of the control rabbit.

The course of the antibody activity in serum and aqueous humor is shown in Figure 5. The data presented in this graph are, again, mean values of the whole group, of nine rabbits. The activity of the serum increased slightly after the second infection. The aqueous humor of the right, reinfected eyes had much higher titers than the sera, and even the aqueous humor of the left, previously normal eyes reached higher levels of

activity than the sera. Observations were discontinued after the third week, since the inflammation of the eyes had decreased to such a degree that accurate determination of the proteins in the aqueous humor was no longer possible.

In histological preparations of reinfected eyes of animals killed 32 days after the second inoculation, signs of a chronic uveitis were apparent. The character of this inflammation was in some cases diffuse and in others was confined to circumscribed foci. These foci consisted of a few epithelioid cells with many pigmentophores at the periphery (Fig. 6). Except for a few plasma cells in the iris, practically no pathological changes were noted in the previously normal, left eyes removed at the same time.

3. *Primary Infection Combined with Local Irritation.*—In this experiment five albino rabbits were used. In each case, 10 days prior to the intraocular infection, the animal was anesthetized with ether and an incision made at the limbus of the right eye. Through this wound a tuft of 10 to 20 sterile caterpillar hairs was introduced into the anterior chamber with a small forceps. Ten days later these eyes were inoculated intravitreously with about 20,000,000 living leptospires. In the control rabbit caterpillar hairs

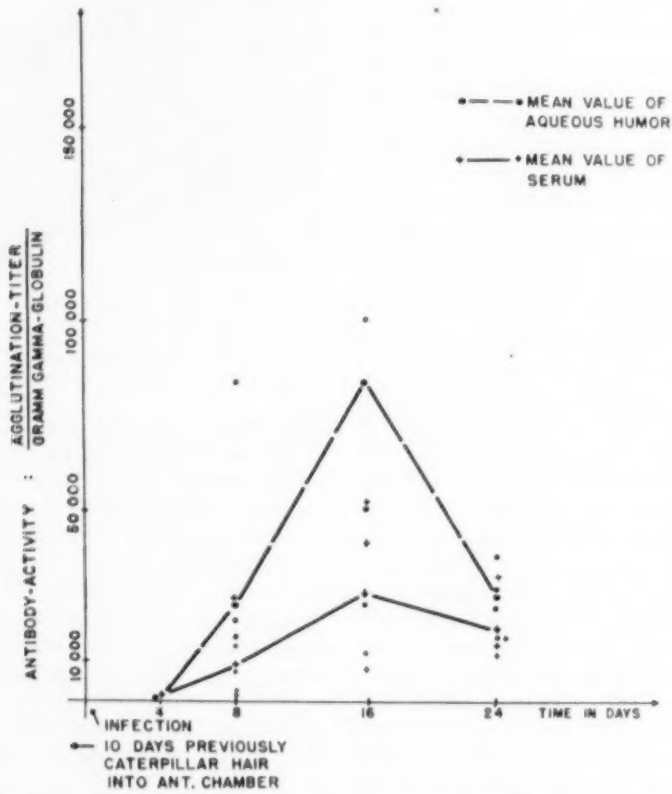
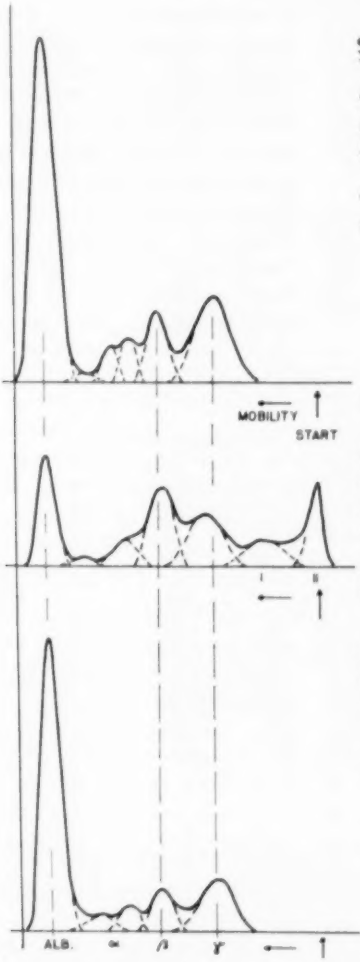


Fig. 7.—Course of antibody activity of serum and aqueous humor after primary intraocular infection with Leptospira pomona and previous implantation of caterpillar hair into the anterior chamber.

were implanted into the right eye. This eye was enucleated after 10 days, at which time the normal, left eye was infected with the same amount of organisms as the whole group.

larization of the cornea, were noted. However, at the time of the leptospiral infection, 10 days after the implantation of the caterpillar hair, the eyes were still greatly inflamed. As a consequence, the response to



SERUM

	REL.%	GR.%
ALBUMIN	57.0	
α-GLOB.	10.0	
β- "	11.3	
γ- "	21.6	1.56
TOTAL PROTEIN		7.25
AGGL.TITER		1:20 000
TITER/γ-GLOBULIN		13 000

UVEAL EXTRACT

	REL.%	GR.%
ALBUMIN	40.7	
γ-GLOB.	21.6	1.0
TOTAL PROTEIN		4.26
AGGL.TITER		1:40 000
TITER/γ-GLOBULIN		40 000

AQUEOUS HUMOR

	REL.%	GR.%
ALBUMIN	64.6	
α-GLOB.	9.9	
β- "	9.6	
γ- "	15.9	0.42
TOTAL PROTEIN		2.65
AGGL.TITER		1:10 000
TITER/γ-GLOBULIN		24 000

I+II UNKNOWN FRACTIONS WITH LOW MOBILITY

Fig. 8.—Electrophorograms of serum, aqueous humor, and uveal extract of rabbit 28 after primary intraocular infection with Leptospira pomona and previous implantation of caterpillar hair.

After the introduction of the caterpillar hairs all rabbits showed an intensive inflammatory response with fibrinous exudate in the anterior chamber. After one week, the inflammation began to diminish slowly, and signs of organization of the exudate around the hair, accompanied by superficial vascu-

the infection was violent: The anterior chamber became filled with fibrin, the cornea steamy, and the vessels of the iris extremely distended. This picture did not change appreciably for three weeks, and the eyes were markedly inflamed when all rabbits of this group were killed and the eyes removed for

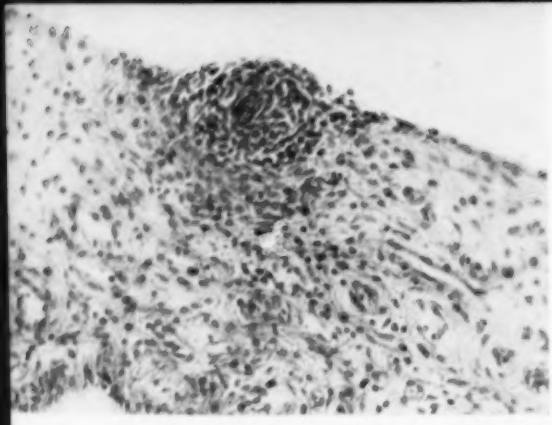


Fig. 9 (Rabbit 29).—Ten days after implantation of caterpillar hair. Small granuloma around hair in stroma of iris; little cellular reaction.

histological study. The left eye of the control rabbit (without caterpillar hair) also had an intensive fibrinous reaction, but after two weeks the inflammation was much less pronounced.

The antibody response in serum and aqueous humor is shown in Figure 7. As early as the fourth day after the infection very low titers were detected in all sera except that of the control rabbit. The aqueous humor samples were negative at this time. Eight days after the infection the titers in both serum and aqueous humor were strongly positive, and the antibody activity of the aqueous humor appears in the graph to be three times as high as that of the serum. However, since the result of one experiment raises the mean value of the antibody activity of the aqueous humor to this level, not much significance can be attached to this ratio. The wide spread should also be kept in mind when considering that on the 16th day the antibody activity ratio of the two fluids

TABLE 5.—*Primary Aqueous Humor in Primary Infected Eyes with Caterpillar Hair*

Rabbit No.	Days After Infection	Observed Titers		Titer/Gm. Gamma Globulin			Gamma Globulin Content, Gm./100 Cc.	
		Serum	Aqueous	Serum	Aqueous	Ratio *	Serum	Aqueous
25	8	16,000	8,000	14,700	16,000	1.09	1.09	0.49
26	8	32,000	14,000	26,000	83,000	3.20	1.20	0.77
27	8	3,200	16,000	2,500	21,600	8.64	1.26	0.74
28	8	3,200	8,000	2,100	8,000	3.80	1.52	1.02
25	16	32,000	8,000	42,000	50,000	1.19	0.75	0.16
26	16	16,000	16,000	12,000	26,000	2.17	1.35	0.61
27	16	64,000	32,000	52,000	160,000	3.08	1.23	0.20
28	16	16,000	64,000	8,400	100,000	11.90	1.90	0.60
25	24	20,000	10,000	16,000	16,000	1.00	1.26	0.61
26	24	20,000	20,000	14,300	28,000	2.00	1.40	0.72
27	24	40,000	20,000	33,000	37,000	1.12	1.22	0.54
28	24	20,000	10,000	13,000	24,000	1.85	1.56	0.42
Average				17,000	47,000	3.45	1.31	0.57

* Ratio: $\frac{\text{antibody activity of aqueous humor}}{\text{antibody activity of serum}}$

Fig. 10 (Rabbit 26).—Twenty-four days after primary leptospiral intraocular infection; 34 days after implantation of caterpillar hair. Antibody activity of serum, 14,300; of aqueous humor, 28,000. Extensive granuloma with fragments of caterpillar hair, giant cells, and dense infiltration by plasma cells, polymorphonuclear neutrophiles, and many lymphocytes. Plastic iritis.

seemed essentially unchanged, although the absolute titers were much higher. Twenty-four days after infection the average titers were still high, but the antibody activity of the aqueous humor was reduced to only about twice that of the serum. Table 5 shows the ratio between antibody activity of aqueous humor and that of serum to be 3.45, approximately the same value as in Tables 3 and 4. When Experiment 28, with the unusually high ratio of 11.9, is omitted, the average is reduced to 2.65. An extract of uveal tissue showed activity three times as

high as that of serum at the time when the activity of the aqueous humor was less than twice as high (Fig. 8).

The histological picture of the right eye of the control rabbit indicated a rather mild reaction to caterpillar hair at 10 days. Typical giant cells, a few polymorphonuclear neutrophiles, and many lymphocytes and plasma cells surrounded the foreign bodies (Fig. 9). The cellular response seemed more pronounced in corneal and conjunctival tissue than in the uveal structures. In the left eye of the control rabbit the high dosage of leptospires produced a severe reaction, consisting of diffuse plasma cell infiltration of iris, ciliary body, and, particularly, the ciliary processes, 22 days after the infection. Leptospiral infection in eyes irritated by the caterpillar hair resulted in a form of plastic inflammation with dense infiltration of the entire uveal tissue, with the exception of the posterior part of the choroid. Accumulated plasma cells, lymphocytes, polymorphonuclear neutrophiles, and giant cells formed small nodules around the caterpillar hair (Fig. 10).

COMMENT

Primary intravitreal infection with L. pomona in rabbit eyes gave rise to an acute anterior uveitis of about one week's duration, followed by a chronic inflammation lasting three to six weeks. Reinfection again produced an acute attack of iritis, which began to subside after one week; in most cases recovery was complete, but in two instances a chronic uveitis ensued. A combination of leptospiral infection with local irritation by caterpillar hair resulted in a plastic granulomatous anterior uveitis. Leptospires could be cultured out of the infected eyes only during the first week. No growth occurred when the various media were inoculated with samples of aqueous humor obtained at later periods. Dark-field examinations of these samples and kidney cultures from the animals were negative without exception.

Despite the absence of any signs of systemic infection, antibodies were detected first in the serum, although in small amounts. In primary infection with low dosage, agglutination titers were measurable after eight days; when higher dosages of the organisms were introduced, together with caterpillar hair as an "adjuvant," low agglutination titers were determined in the serum after four to six days. By the 10th day of the infection antibodies in moderate amounts appeared also in the aqueous humor of the diseased eyes, although with a few exceptions the titers were lower than the serum titers. The antibody response of the individual animals varied to a marked degree; as a rule the more inflamed eyes showed higher agglutination titers. From the 14th day on, however, the antibody activity (i. e., the agglutination titer per gram of gamma globulin) in the aqueous humor of the infected eyes surpassed that of the serum. The average antibody activity ratio of aqueous humor to serum ranged from 2.5 to 3.7 in the entire experimental group of primary infected eyes. On the other hand, data on antibody activity in the first and second aqueous humor of the normal eyes *versus* serum give a ratio of 0.3 (Tables 1 and 2), seeming to indicate that in a normal eye only about a third of the total amount of antibodies present in the serum passes the blood-aqueous barrier. In view of this low ratio in normal eyes, the data on the infected eyes takes on an even greater significance. Aqueous humor of inflamed eyes and secondary aqueous humor of normal eyes contain all protein fractions of the serum, including the gamma globulins, in approximately the same distribution.[6] If the proteins of the aqueous humor were but a simple dilution of the serum and the presence of antibodies, therefore, due only to nonspecific leakage of proteins through the blood-aqueous barrier, the antibody activity would be the same as in the serum. For instance, a serum sample with an agglutination titer of 1:20,000 and a gamma globulin content of 0.75 gm. per 100 cc. would have an antibody activity of 25,000. If the gamma globulin content of the aqueous humor in this case were 0.25 gm. per 100 cc. and the antibody activity the same as that in the

serum, the titer would be 1:6250. If, on the other hand, a titer of 1:12,000 was determined in the sample, then the antibody activity of the aqueous humor would be 48,000, or nearly twice as high as that of the serum, although the actual titer of the latter was higher. On the basis of such results a local antibody formation is the logical assumption. Similar evidence was obtained from the experiments in which caterpillar hair was implanted prior to the intraocular infection. The experimental lesion produced by insertion of caterpillar hair in the rabbit eye resembles that of ophthalmia nodosa in man.§ However, previous attempts to produce such an uveitis experimentally met with little success.[13] In the present short series the average antibody activity ratio was 3.45 (in one rabbit the antibody activity of the eye was 11 times that of the serum). The caterpillar hair as a local irritant and granuloma-producing factor apparently accelerated the antibody formation and augmented the severity and duration of the inflammation. Fischel and co-workers[13] suggest as the mechanism of action of Freund's adjuvant[14] that the granulomatous tissue developing around the killed tubercle bacilli promotes increased antibody formation. Such a theoretical explanation seems to be applicable to the present experiments with caterpillar hair, since dense plasma cell infiltration of the iris and ciliary body was the predominant pathological change. The appearance of antibodies in the aqueous humor coincident with the appearance of infiltrating plasma cells in the uveal tissue (10 to 14 days after the infection) would support the hypothesis of a probable correlation between local antibody formation and plasma cell infiltration. The fact that densely infiltrated eyes always showed much higher antibody activity than eyes with signs of mild inflammation provides further evidence for such a correlation. In addition, the experiments with caterpillar hair revealed augmentation of antibody activity (particularly in extracts of uveal tissue) concurrent with an intensive cellular reaction. The delay in the appearance of antibodies in the eye following the intraocular infection and the definite correlation between antibody activity in the eye and plasma cell infiltration of the uveal tract suggest that antibody formation originates at unknown extraocular sites in the organism. A "leakage" of antigen out of the eye would explain this early presence of agglutinins in the serum. The plasma cells and lymphocytes which invade the uveal tract in the later stage of the infection may be the cause of the accumulation of specific antibodies in the eye in the presence of considerable amounts of antigen.

Whatever the mechanism of antibody formation may be, the results of this and previous studies demonstrating local antibody accumulation[16] and a higher antibody activity in aqueous humor of infected eyes with signs of uveitis as opposed to that of the serum of such animals provides an additional tool for the differential diagnosis of bacterial uveitis. It seems advisable, therefore, to apply this technique, previously confined to the identification of tuberculous lesions and periodic ophthalmia in horses, to other endogenous types of human uveitis in which bacterial etiology is suspected. Even low specific antibody titers in the aqueous humor of infected eyes may be considered significant in view of the very low protein and gamma globulin contents of human aqueous humor of normal and inflamed eyes.

SUMMARY AND CONCLUSION

Primary intraocular infection with Leptospira pomona in rabbits produces an uveitic reaction, with an acute phase lasting about one week and an ensuing subchronic course. Reinfected eyes are, to a great extent, immune but may respond to the second inoculation with the formation of small iris nodules.

Antibody formation in the experimental infections seems to start at unknown extraocular sites, since the first measurable titers are in the serum (10-day interval). Later, 14 days after infection, a local antibody accumulation takes place, and from that time on the antibody activity of the aqueous humor—that is, the reciprocal of agglutinin

§ References 11 and 12.

titers per gram of gamma globulin (measured by means of paper electrophoresis)—exceeds that of the serum by about 3:1. This antibody production is accelerated when an adjuvant, caterpillar hair, which produces a severe plastic iridocyclitis, is implanted in the anterior chamber.

Histologically early hyperemia of the iris and invasion of the vitreous by polymorphonuclear neutrophiles is succeeded from the 10th day on by an infiltration of the uveal tissue with plasma cells and lymphocytes, which gradually subsides after four to six weeks.

The appearance of antibodies in the aqueous humor coincident with the appearance of infiltrating plasma cells and lymphocytes in the uvea suggests a close correlation between the accumulation of these cell elements and high antibody activity.

The results of this study confirm earlier reports on periodic ophthalmia in horses and experimental tuberculous uveitis in rabbits, where strong evidence for local antibody formation was presented. Determination of antibody activity in aqueous humor and serum therefore seems to be a potentially useful tool for the etiologic diagnosis of uveitis.

Dr. Ludwig von Sallmann and Dr. Seymour P. Halbert gave advice and counsel in this study. Mr. Charles L. Remington, Osborn Zoological Laboratory, Yale University, determined the caterpillar species, and Col. Robert H. Yager and Lieut. Col. Chester A. Gleiser, Veterinary Division, Army Medical Service Graduate School, provided the leptospires.

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Bilateral Homonymous Hemianopsia

Relationship to Thrombosis of the Basilar Artery

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Numerous reports in the literature concern bilateral homonymous field defects. In a few of the reported cases the right and left homonymous defects developed simultaneously. There is, however, a paucity of references attributing such field defects to occlusive disease of the basilar artery. Neither Walsh [1] nor Duke-Elder [2] mentioned this association. Thrombosis of the basilar artery is not common, and when such an event does occur the patient usually does not survive; occasionally it is not immediately fatal, as has been recently reported by Siekert and Sayre.[3]

REVIEW OF THE LITERATURE

The earliest report of bilateral homonymous field defects is probably that of Bramwell and associates,[4] in 1915. Their patient exhibited sudden onset of bilateral homonymous field defects with preservation of central vision. This case is unusual in that the authors first saw the patient in 1888, two years after the onset of visual disturbances, and were able to observe him for approximately 25 years, until his death, at which time necropsy revealed bilateral infarction of the occipital lobes.

McDonald [5] reported a case of similar nature except that the field defects did not develop simultaneously. Necropsy was not performed. The cause of the field defects was thought to be occlusion of both calcarine arteries. More recently, Ross [6] and Steiner [7] each reported a case in which the homonymous defects were simultaneous in onset. They considered the cause to be occlusive disease of both posterior cerebral or calcarine arteries. Necropsy was not done in either case.

Walsh [1] said that bilateral homonymous field defects occur simultaneously in a minority of cases. He also expressed the opinion that complete obstruction of the basilar artery causes death within a short time. Patients who survive occlusive disease of the basilar artery perhaps do not have complete thrombosis but have only partial occlusion of the vessel, or thrombosis involving only its terminal portion as it bifurcates into the two posterior cerebral arteries. The basilar artery gives rise to small penetrating arteries that supply the pons, and pontine infarction is to be expected in complete thrombosis of the basilar artery. Massive infarction of the pons is fatal, although small infarcts may occur without interfering with vital respiratory and cardiac function.

Kubik and Adams [8] described the symptoms of thrombosis of the basilar artery on the basis of a study of seven patients who survived the incident. The diagnoses were made on what they considered to be adequate clinical evidence. The onset was sudden and was not preceded by "tangible causal factors." Headache was usually the first symptom, followed by dizziness, confusion, or coma. Difficulty in speaking and unilateral paresthesias occurred in a large proportion of cases. Common findings were pupillary abnormalities, disorders of ocular movement, facial palsy, hemiplegia, and quadriplegia. Bilateral ex-

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tensor plantar reflexes were present in most of the cases reported by the authors.

Biemond [9] reported on four patients who had occlusion of the basilar artery; the diagnosis in all instances was proved later by necropsy. One of the patients complained of dimness of vision, and temporary homonymous field defects were demonstrated.

French and Haines [10] reported an interesting case of occlusion of the basilar artery in which there was limitation of extraocular movements suggestive of anterior internuclear ophthalmoplegia. Such ophthalmoplegia results from a lesion of the brain stem. It is due most frequently to demyelinating disease, but does occur in vascular occlusive disease, as has been recently reported by Cogan and associates.[11]

Pupillary abnormalities have been reported in a number of cases. In the aforementioned case of French and Haines, unilateral Horner's syndrome was present, probably due to involvement of the brain stem. Gross and coworkers [12] reported two cases of thrombosis of the basilar artery in which the only signs were small, nonreacting pupils.

In a recent paper, under the title "Bilateral Homonymous Hemianopsia," Reese [13] discussed cortical blindness arising from a variety of causes. In one of the cases, he suggested the diagnosis of embolism, or thrombosis, at the junction of the basilar and posterior cerebral arteries, with extension beyond the posterior communicating arteries. Necropsy was not done; so the diagnosis was not confirmed. He mentioned a case, not personally observed, in which a thrombus was found at this location. He did not state whether or not field defects were present in this case, but he did say that closure of the basilar artery usually fails to produce visual field defects because of the blood supply to the posterior cerebral arteries through the posterior communicating arteries. It was his impression, because of the absence of changes in the brain stem in all but two cases, that the posterior cerebral arteries were involved beyond the branches supplying the brain stem.

REPORT OF CASES

We wish to report three cases seen recently at the Mayo Clinic in which bilateral homonymous field defects and other ocular signs developing simultaneously were thought to be due to thrombosis of the basilar artery. The diagnoses were made on clinical grounds in all cases. Death occurred in one case, and the presence of thrombosis was confirmed at necropsy.

CASE 1.—A 59-year-old farmer was well until the day before admission to the hospital, in July, 1953. He had arisen from bed early the previous morning to watch a thunderstorm, which was accompanied by much lightning. He suffered no ill effects at that time, but later in the morning, after dressing himself and feeding the chickens, he suddenly became blind. He was somewhat confused but remembered the lightning and attributed his blindness to this.

On admission he complained only of the visual trouble. Cooperation was good, although he appeared mildly confused; although fully aware that something was wrong with his eyes, he was not unduly concerned. The blood pressure was 186/98. Neurologic examination revealed slightly slurred speech and a bilateral Babinski sign. It was difficult to be certain that he retained any vision. He apparently saw movements of the hand vaguely in the far periphery of the right half-fields. The pupils were small and reacted promptly to light. Ocular rotation was normal except for horizontal nystagmus on gaze to either side. The fundi were normal except for arteriolar changes typical of mild chronic hypertension.

Results of routine laboratory studies were normal. A roentgenogram of the head showed nothing abnormal. A 24-hour specimen of urine contained no arsenic and only 0.03 mg. of lead per 100 cc., an amount considered to be of no significance.

Because of the apparent total loss of central vision and the impossibility of demonstrating preservation of macular vision, the possibility of a lesion involving the chiasm or optic nerves was considered at first. However, the normal pupillary reactions and the absence of changes in the roentgenogram of the head were against the presence of such a lesion, and the final diagnosis was hypertensive cerebral vascular disease with infarction of the occipital lobes. Partial occlusion of the basilar artery or occlusion of only the terminal portion was thought to be the specific cause of his difficulty. Treatment consisted of inhalation of 5% carbon dioxide and 95% oxygen for 5 to 10 minutes each hour. No improvement in vision occurred, and after several weeks he was dismissed from the hospital.

This patient is still living but has been committed to a state mental institution because of his confusion. He was examined recently by one of us. The findings were essentially unchanged; the pupils still reacted normally, and the optic nerve heads had a good pink color.

Case 2.—A 69-year-old woman was in good health until about three weeks prior to admission to the hospital. While out in the woods hunting wild grapes, she suddenly felt lightheaded, had a tendency to fall backward, and had to be supported by her husband. She also noted numbness of the left side of her face and apparently experienced homonymous hemianopsia, since she described half of her vision as being blurred. The symptoms were

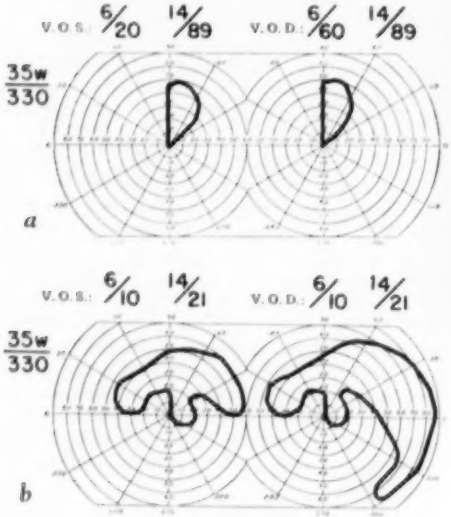


Fig. 1 (Case 2).—Visual fields as plotted on Nov. 18 (*a*) and Dec. 4 (*b*).

of brief duration, lasting only three to five minutes, but they recurred several times during the next three weeks.

On her admission to the hospital, in November, 1953, the blood pressure was 210/110. Objective neurologic and ophthalmologic findings were normal. The visual fields were normal to confrontation testing, and she was able to read small print (14/21 on the A. M. A. reading chart). The impression was that she was having attacks of focal cerebral disturbance, probably of vascular origin.

In spite of inhalation of carbon dioxide and oxygen, the attacks continued. Bilateral homonymous field defects developed two weeks after admission. She then was able to read only 14/89 on the A. M. A. chart with either eye and appeared to be visually disoriented. Left internuclear ophthal-

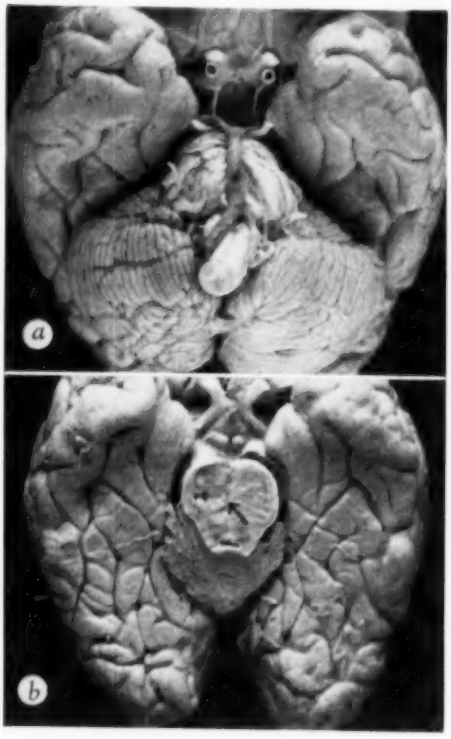
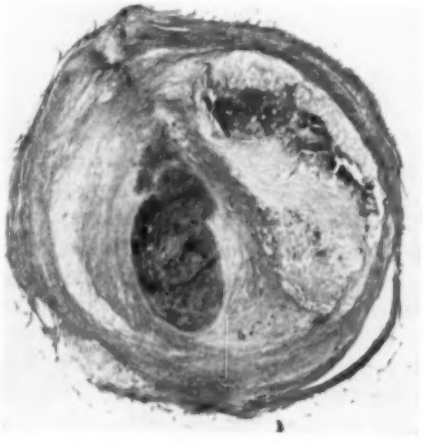


Fig. 2 (Case 2).—(*a*) Thrombus in right vertebral and basilar arteries; (*b*) infarct in pons.

Fig. 3 (Case 2).—Cross section of basilar artery showing degree of thrombosis; Van Gieson elastic tissue stain; × 14.



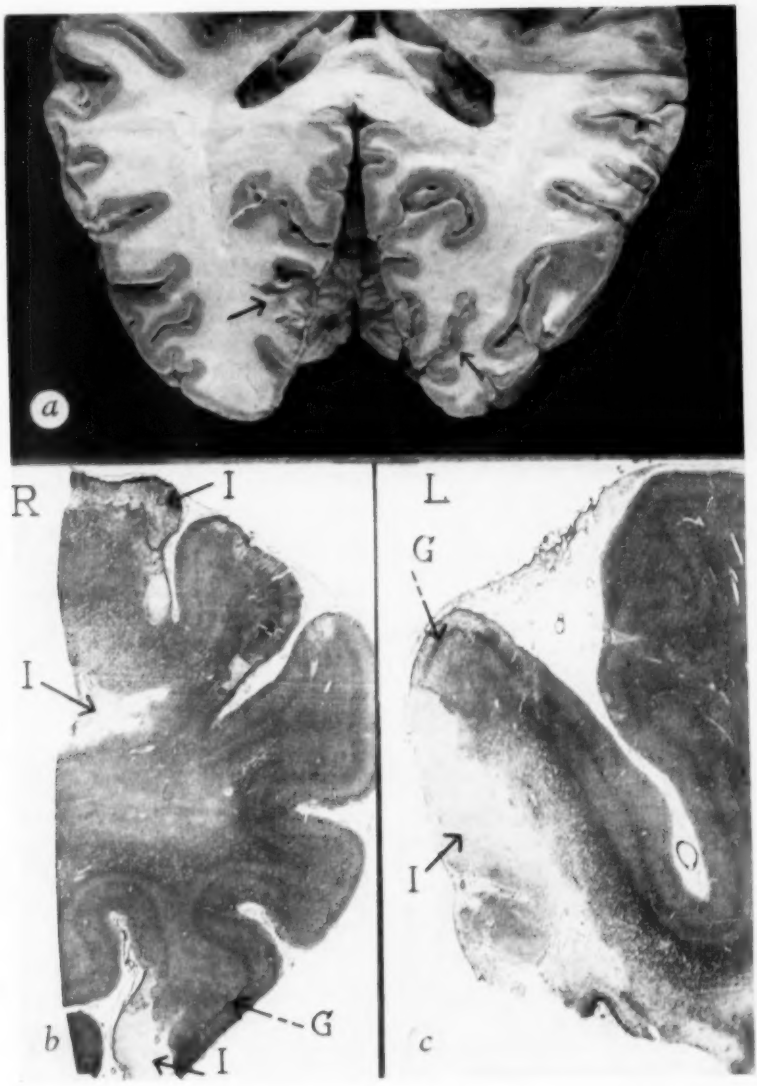


Fig. 4 (Case 2).—Infarcts in occipital lobes. (*a*) Gross appearance; (*b* and *c*) sections through medial aspect of both occipital lobes. *R* indicates right; *L*, left; *G*, line of Gennari; *I*, infarcts. Hematoxylin and eosin; × 3.

moplegia was also present. On gaze to the left, adduction of the right eye was nil, while abduction of the left eye was normal; nystagmus of the left eye developed after rotation in this direction. The visual fields at that time (Nov. 18) are seen in Figure 1*a*. The optic discs appeared normal.

The impression was that thrombosis of the terminal portion of the basilar artery, or perhaps partial thrombosis of the entire vessel, had occurred. Anticoagulant therapy with heparin and bishydroxycoumarin (Dicumarol) was begun immediately. Vision gradually improved, and she soon became able to get about the hospital unaided. The visual fields on Dec. 4 are seen in Figure 1*b*.

She was dismissed from the hospital on Dec. 14 and was followed periodically at the clinic as an outpatient. On Jan. 14, 1954, she came to the clinic unaided. The ocular fundi and the visual fields were

unchanged. The internuclear ophthalmoplegia was still present and gave rise to an annoying diplopia. On Jan. 18 she became unable to speak and, although fully conscious, was unable to use her extremities. She was readmitted to the hospital, where she responded to questions by movements of of her head. She became unconscious on the following day and died on Jan. 23.

At necropsy the immediate cause of death was found to be massive pulmonary embolism. The cerebral vessels showed sclerosis Grade 4 on the basis of 1 to 4. The sclerotic basilar and vertebral arteries had distorted the brain stem somewhat. The right vertebral artery, the larger of the two, was filled with an old thrombus that extended up into the basilar artery (Fig. 2*a*). Infarction of the pons was present (Fig. 2*b*). A cross section of the thrombosed basilar artery is seen in Figure 3. Infarcts were present in both occipital lobes (Fig. 4*a* and *b*).

Both the internuclear ophthalmoplegia and the bilateral homonymous field defects developed while the patient was under observation in the hospital, so that no question existed about their simultaneous and sudden onset. Necropsy confirmed the clinical impression of thrombosis of the basilar artery. Although both posterior communicating arteries were present and patent, they were apparently unable to supply adequate collateral circulation for the preservation of the posterior portion of the cerebrum.

Case 3.—A 70-year-old farmer, previously in good health except for cardiac infarction in 1951, was hospitalized in February, 1954. Six days prior to admission he suddenly had become wobbly on his feet and had felt weak; his eyes had not focused properly and had appeared to turn out. He was seen by his family doctor, who diagnosed his condition as a stroke and advised rest in bed. He made a rather quick recovery and five days later was well enough to play his violin. The following day he complained of decreased visual acuity and double vision. He was brought to the hospital on the same day.

The outstanding observation was that periodically he would lapse into coma and exhibit Cheyne-Stokes respiration. After a brief period of unconsciousness he would awake and be remarkably clear mentally. Neurologic examination showed slight facial weakness on the right and a bilateral Babinski sign. Visual acuity was greatly reduced. He could read only 14/89 on the A. M. A. chart with the right eye and 14/224 with the left. Confrontation fields showed bilateral contraction, which appeared to be more pronounced inferiorly than elsewhere. It was difficult for him to find the letters, which made results of testing of central vision somewhat unreliable. Anterior internuclear ophthalmoplegia was present on gaze to the right. On looking to the right, the out-turning eye developed nystagmus, while the in-turning eye failed to adduct. The ocular fundi were normal. Results of routine laboratory tests were within normal limits. A roentgenogram of the head disclosed nothing abnormal.

A diagnosis of occlusive disease of the basilar artery was made, and anticoagulant therapy was started. His ocular findings showed little change, but his general condition improved, so that he was soon able to sit up for meals and take short walks in the hall with the aid of the nurse. Five days after admission he described tingling in his left arm and hand and was afraid he was getting a stroke. During his stay in the hospital he never appeared fully to grasp the fact that he was almost blind and had to be led around. Visual fields were plotted one week after admission (Fig. 5).

The patient was examined periodically at the clinic. Five months later the internuclear ophthalmoplegia had disappeared, and he no longer noted double vision. He often bumped into objects, such as a door facing. He was able to read 14/28 print with each eye with difficulty. The perimetric fields

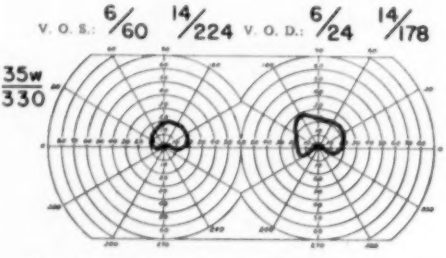


Fig. 5 (Case 3).—Visual fields as plotted one week after patient's admission to hospital.

were essentially unchanged from those plotted in the hospital. The ocular fundi were still normal. His general condition remained about the same as it was on dismissal from the hospital.

Although the diagnosis in this case has not been proved, the great similarity to Case 2 from the standpoint of bilateral homonymous field defects and internuclear ophthalmoplegia appears to justify the diagnosis of thrombosis of the basilar artery. The rapid fluctuations of consciousness and the Cheyne-Stokes respiration exhibited by this patient were also present in the case reported by Siekert and Sayre.[3]

COMMENT

These three patients all presented similar findings, namely, sudden simultaneous onset of bilateral homonymous field defects, ocular signs of involvement of the brain stem, plan-

tar extensor signs, transient ataxia, and focal signs, such as facial palsy, weakness of extremities, and paresthesias. All of them exhibited to some degree a phenomenon mentioned by Duke-Elder,[2] consisting of subjective unawareness of the visual disability. The patients apparently were "blind to their blindness," a symptom commonly seen in bilateral destructive lesions of the visual cortex. This naturally imposes difficulty in testing vision, especially the visual fields. Thus we were never able to be certain of the exact visual status in the first patient. Perhaps this confused state and the visual disorientation accounted for the poor central vision noted in all patients. The central vision did appear to improve with time, as was well demonstrated in the second patient. Theoretically, central vision should be spared, since it is generally accepted that the middle cerebral arteries supply the occipital poles, and macular sparing should occur in bilateral homonymous defects due to lesions in the basilar system.

SUMMARY AND CONCLUSIONS

Three patients who had bilateral homonymous field defects, believed due to occlusive disease of the basilar artery, were studied at the Mayo Clinic. The diagnosis was made clinically in all the patients and was proved at necropsy in the one patient who subsequently died. From the ophthalmologic standpoint, the sudden and simultaneous development of bilateral homonymous field defects and signs of a lesion in the brain stem, such as nystagmus and internuclear ophthalmoplegia, would appear to be diagnostic of occlusion of the basilar artery. It is important to realize that patients occasionally survive thrombosis of the basilar artery and present ocular complaints as their major problem.

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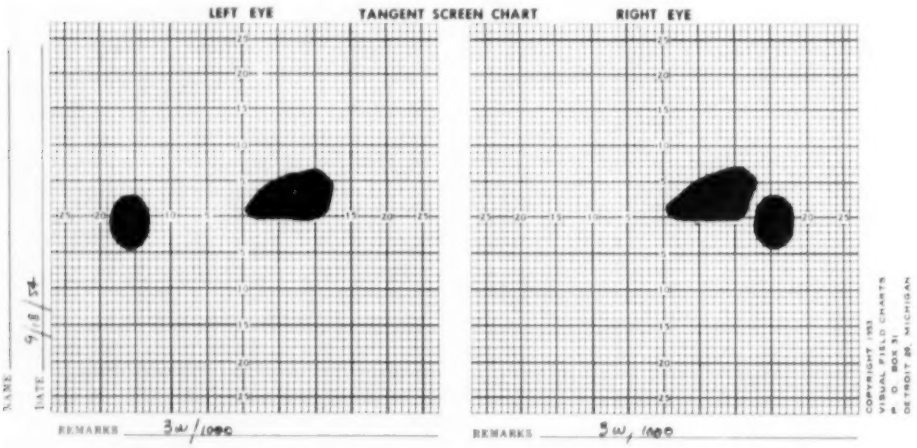
Registry of Interesting Cases

It is the purpose of this section to record cases on the basis of interest only. No complete review of the literature is attempted and no claim for priority is implied except when specifically stated. Publication of case reports is subject to the concurrence of the Editorial Board on the interesting aspects of the case. It is requested that the reports submitted be as brief as is consistent with inclusion of the pertinent facts, bear a title that is as specific as possible, be prefaced by a paragraph that states why the author feels the case is of interest and be accompanied with photographs whenever possible.

PERMANENT HOMONYMOUS MIGRAINE SCOTOMATA

The subjective manifestations of the ophthalmic migraine syndrome which precede the actual headache are protean in form, yet probably have a common vascular pathogenesis. These variously described visual auras are largely transient, usually lasting less than half an hour, although cases have been reported in which a permanent field defect remained. This is described as a rare event by Duke-Elder,



Field defect charted on tangent screen Sept. 18, 1954; test object $\frac{3}{1000}$ white.

usually occurring only in severe cases of long standing, suggesting that the spasmodic disturbance had left structural defects.* The following case was considered to be of unusual interest because of the association of permanent scotomata with otherwise mild migraine.

REPORT OF CASE

The patient, a 31-year-old physician, gave a history of migraine attacks two or three times a year, invariably preceded by a scintillating scotoma, lasting 10 to 15 minutes and followed by a mild headache, which was usually completely relieved by 10 grains (0.6 gm.) of acetylsalicylic acid. No flushing, nausea, vomiting, prostration, or ophthalmoplegia had occurred. The family history was negative for migraine and other paroxysmal disorders.

* References 1 and 2.

On Feb. 2, 1953, the patient developed his usual visual aura, in this case a right homonymous scotoma, for which he took the usual 10 grains of acetylsalicylic acid. In this instance, however, the headache was incompletely prevented and codeine, ½ grain (30 mg.), was taken for relief. Likewise, the scotomata did not fade away in the accustomed fashion, but persisted in the form of a complete right homonymous hemianopsia, which spared the maculae. This field defect was repeatedly charted during the ensuing weeks, during which time it gradually diminished in size and became less bothersome to the patient. The extreme periphery of the scotoma was relative in density for a time, making reading almost impossible, owing to the distortion of print to the right of fixation. This fortunately subsided, leaving a dense paracentral scotoma in each right superior quadrant (Figure); this defect has persisted to the present, one and a half years since the onset. At present the patient has adapted to his disability in rapid reading by a slight head tilt toward the left shoulder, while effectively rotates his scotoma to the line of print above that of fixation.

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News and Comment

EDITED BY DR. W. L. BENEDICT

ANNOUNCEMENTS

Robert J. Masters Award Fund.—Establishment of the Robert J. Masters Award Fund in the Indiana University School of Medicine, as a recognition of Dr. Masters' contributions to medical education during the past thirty years as a member of the faculty, has been announced by his students and friends.

A bronze plaque bearing the inscription

Robert J. Masters Award Fund
to Honor
Robert J. Masters, M.D.
Distinguished Leader and Chairman
of the
Department of Ophthalmology
1943-1954
Established by His Students and Friends

will be placed in the offices of the Department of Ophthalmology, where Dr. Masters has served as a member of the staff since 1925, shortly after receiving his M.D. degree from Indiana University.

In addition to the plaque, Dr. Masters has been presented with a memorial volume containing congratulatory messages from students and associates in all parts of the country.

Dr. Masters, who retired as Chairman last year, continues as Professor of Ophthalmology and maintains his active interest in the Department and his practice. Under his leadership the Department of Ophthalmology and its eye clinics attained national recognition in teaching, service, and research. Dr. Masters has been prominent in many professional organizations and has filled important offices, including that of Chairman of the A. M. A. Section on Ophthalmology.

Dr. Fred M. Wilson, who has succeeeded Dr. Masters as Chairman of the Department, has announced that the Award Fund will be used for graduate students in the Department of Ophthalmology as recognition of unusual excellence and achievement.

VITAL STAINING OF CONJUNCTIVA AND CORNEA

Review of Literature and Critical Study of Certain Dyes

LIEUT. COL. JACK W. PASSMORE
and
COL. J. H. KING Jr. (MC), U. S. Army

Staining of the conjunctiva and cornea has been practiced for years as a diagnostic aid. Campbell and Boyd [1] note that sodium fluorescein has been used by ophthalmologists for initial detection of breaks in the continuity of the epithelium of the cornea since 1882 (Pflüger, 1882; Straub, 1888). Berliner [2] credits Knüsel and Von Miller for much of the original work in vital staining of the conjunctiva and cornea. More recently other authors have evaluated certain stains employed for specific uses; however, there is an obvious dearth of material concerning this subject.

The importance of staining in ophthalmology has stimulated us to review the literature and to evaluate dyes which we have employed in our clinic.

THEORIES OF STAINING

There are numerous theories attempting to explain staining. The probability is that staining is both a chemical and a physical phenomenon. The physical theory accounts for the penetration of the dye into the interior of the tissue by capillarity and osmosis, adsorption, or absorption, or a combination of these. The chemical theory provides that tissues have definite chemical affinities which are satisfied by the chemical affinity of the dyes. How often staining of living cells (vital staining) is accomplished in the eye is not known. It is known that it is possible to stain the living cells of nerves and the corneal stroma. However, the staining of the precorneal film, of superficial lesions—especially with breaks in the corneal epithelium—of devitalized elements, and of subepithelial tissue is important.

DYES EMPLOYED

Conn [3] classifies dyes in two groups, the natural and the artificial. The former has few important representatives from the biological standpoint. Carmine, a cochineal derivative, has been employed as an ophthalmic stain. The artificial, or coal tar, dyes, which number over 100, may be considered as derivatives of the hydrocarbon benzene (C_6H_6). Conn defines a dye as an organic compound which contains chromophoric and auxochromic groups attached to benzene rings, the color being attributable to the chromophore, and the dyeing property to the salt-forming auxochrome. In ophthalmology greatest attention has been focused on certain water-soluble dyes that have proved most successful in staining the conjunctiva and cornea. These include fluorescein (Na salt), merbromin (Mercurochrome), rose bengal (Na salt), methylene blue (chloride), polychrome methylene blue, azure II, cresyl blue, Nile blue sulfate, and scarlet red.

Dye nomenclature is extremely confusing in the literature, and many dyes have several synonyms. Those with the same name may vary greatly as to actual dye content, color index, solubility, and impurities. The impurities present in stains affect the solubility,

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the intensity of staining, and the irritability produced when used in the eye.

It is difficult to follow a staining formula noted in the literature and be certain that the solution approaches the identity of the one recommended by an author. In North America, the Biological Stain Commission has done much to remedy this deficiency by specification and certification of biological stains. Fifty-seven stains were certified as of 1950, all submitted by concerns in North America.* The label on each of these dyes contains the percentage of dye content and a certification number.

The above-mentioned facts no doubt explain some of the variable results reported by different authors in evaluating conjunctival and corneal staining.

Factors influencing the stain employed are those which pertain to eye solutions in general. Stability and staining activity are prime requisites. A stain should not produce undue irritation or prolonged discoloration which is visible grossly. It should be free of bacteria, virus, and fungi, and should remain so for the period of time it is used. Many excellent papers have been published on the preparation, sterilization, and preservation of ophthalmic solutions.†

HYDROGEN ION CONCENTRATION

Hind and Goyan [4] cite the importance of the pH in affecting the activity, stability, and irritability of a solution. Since the ideal pH's for these conditions may not coincide, some sacrifice may be necessary. The pH of stains must be that resulting in solubility and good staining properties with the least irritability and greatest stability possible. A dye is termed "acid" or "basic" depending upon the nature of the auxochrome present, and not on the hydrogen concentration. Therefore, acid dyes are often basic in reaction and vice versa.

IRRITABILITY

The irritation produced by a staining solution is modified not only by the impurities present and its pH, but by the preservative added to maintain sterility. Surface anesthesia may be used to lessen irritation, but certain anesthetic agents produce physiological changes and alter the staining characteristics of the conjunctiva and cornea. The devitalizing action of cocaine is desirable when staining subepithelial tissues. Forster [10] uses tetracaine (Pontocaine) prior to staining with rose bengal in the diagnosis of deficient tear formation, but Sjögren [11] warns that the instillation of a local anesthetic before applying the dye has given false-positive results in the diagnosis of this condition.

Although some staining solutions irritate more than others, the irritation noted by the patient is usually proportional to the devitalized area and to the injection of the conjunctival vessels. Anesthesia is not required very frequently in surface staining. If irritation is extreme, immediate irrigation is beneficial, and the staining effect is usually adequate for diagnosis. If the irritation is prolonged, tetracaine and patching may be utilized. In a small series of normal eyes, we have been unable to demonstrate staining following topical application of tetracaine but have demonstrated it often with fluorescein, rose bengal, and methylene blue following use of tetracaine and tonometric instrumentation.

ACTIVITY

The activity of a stain includes its effect, strength and number of applications required to produce the effect, and duration of effect. Staining of the precorneal film requires frequent applications of staining solution if observation is prolonged, as the lids tend to break up its continuity and produce a mottled tear film.

Surface staining for epithelial defects may be accomplished by instilling 1 drop of solution, allowing the lids to close for one minute, and irrigating. When penetration is desired, 2 drops at three to six successive five-minute intervals may be required for the desired re-

* The National Aniline Division-Allied Chemical & Dye Co., New York 6, can supply most stains, 20 of which are pure dyes, prepared especially for vital staining (12 certified).

† References 4 through 9.

sult. Vital staining of nerves and corneal stroma, and even penetration into the aqueous with fluorescein, may be attained in this manner. Irrigation of excess stain may be accomplished with either isotonic saline or boric acid solution.

STERILITY

Sterility is a most important factor in staining solutions, since many eyes which require staining have suffered injuries or have epithelial defects which lower resistance and remove the barrier against infectious organisms. This is true for the most commonly used stains—rose bengal, methylene blue, and, especially, fluorescein. Fluorescein is kept in most ophthalmic clinics, offices, and hospitals. Theodore [6] reports a bacterial study of fluorescein used in a hospital in which all 26 bottles were contaminated with Pseudomonas aeruginosa; 10 of 15 solutions in offices of ophthalmologists were likewise contaminated.

There is much conflict in the literature concerning the sterilization and preservation of eye solutions in general. Sterility is usually obtained by proper preparation, by the addition of an inert, nonirritating bactericidal and fungicidal agent compatible with the staining solution, and by dispensing in a form which minimizes the chance of contamination. The use of bottles with screw tops and individual, disposable plastic droppers, as reported by King,[9] is simple and offers excellent protection. Kimura [12] obtained favorable results with fluorescein-impregnated paper to insure sterility of that stain.

Thiomerosal N. F. (Merthiolate), 1:5,000 to 1:10,000; benzalkonium (Zephiran) chloride, 1:5,000 to 1:10,000, and chlorobutanol, 0.5%, have been employed for purposes of sterility. Benzalkonium is incompatible with the Gifford buffers and results in some precipitation when used as a preservative in fluorescein sodium or buffered rose bengal. Hogan [7] does not consider the incompatibility as affecting the action or use of fluorescein and employs it 1:10,000 in a borate-buffered solution. Benzalkonium is stable and retains its activity. Thiomerosal is sufficiently anti-infective if the other measures mentioned are followed. It is compatible with fluorescein sodium and therefore is the preferred preservative for that stain. It is stable in air and may be autoclaved. It is unstable in sunlight and requires light protection (amber bottle), and it is relatively nonirritating in the strengths recommended. Chlorobutanol is preferred by some [6] because sensitivities are infrequent. A small amount may be added to solutions every two weeks if fresh solutions cannot be obtained.

Preliminary evaluation of the addition of soluble tablets of polymyxin B sulfate (Aerosporin)‡ to fluorescein solution indicates evidence of additional protection against contamination by P. aeruginosa. Further investigations are being made along this line.

INDIVIDUAL STAINS

Aniline, or Coal Tar, Dyes

I. *Xanthene Group* (acid chromophore dyes). Fluorane derivatives (water-soluble sodium salts of fluorescein, merbromin, and rose bengal).§

A. Fluorescein Sodium: A 2% solution is prepared by our pharmacy as follows:

Fluorescein sodium	10
Potassium phosphate	0.95
Sodium phosphate anhydrous	4.05
Sodium chloride	0.18
Thiomerosal (Merthiolate) 1:5000	
qs ad	500

This is filtered through a Büchner filter using hard No. 850 filter paper and is dispensed in 10 cc. amber bottles with a sealed screw cap. It is autoclaved 7 to 10 minutes at 17 to 20 lb. pressure at 250 F. A disposable dropper, previously referred to, is used each time fluorescein is withdrawn from its container. The dropper may be allowed to touch the eye during application, if necessary, as it is discarded after applying the stain to one eye.

‡ Obtained through the courtesy of Burroughs Wellcome & Company, Inc., Tuckahoe, N. Y.

§ These substances are not true dyes but are classified as such because fluorane contains the xanthene ring.

Any staining solution unused two weeks after the seal is broken should be discarded for greatest safety.

Fluorescein is the most satisfactory for surface staining. It stains the precorneal film and demonstrates surface irregularities and changes in epithelial thickness. Ulcerated areas stain green, and foreign bodies are surrounded by a green ring if not epithelized. Early corneal wounds retain the stain for their entire depth. Fluorescein will demonstrate a postoperative filtrating aqueous leak (Seidel test). Mucus and desquamated epithelial cells can be demonstrated. Fluorescein is useful in demonstrating points of pressure in the fitting of contact lenses. Anderson [13] feels that more use of the fluorescent property of fluorescein should be made in studying ocular disorders. He employs light from the violet end of the spectrum as the exciting light, and a yellow filter for active observation in his technique. Campbell and Boyd [2] present a method for assessing the rate of healing in corneal ulcers by comparing the intensity of staining by fluorescein solution with fluorescein-impregnated filter paper under an ultraviolet lamp.

The solution we prepare is active and stable and rarely produces irritability. The application of 1 drop of solution, followed by a 30- to 60-second interval with the lids closed prior to irrigation, is usually sufficient to produce the desired effect. The irrigation of excess stain is easily accomplished, and undesirable discoloration does not occur. Penetration of fluorescein may be produced by more instillations and delayed irrigation if the study of fluorescence in the corneal stroma is desired. The dye can be made to penetrate into the anterior chamber; however, we have had no occasion to utilize this diagnostically.

Sterility is maintained by the technique described above. Repeated cultures on many bottles opened and in use in this clinic for periods up to two weeks have failed to reveal organisms. Typical contamination was simulated on several occasions by making a suspension of the secretion from an infected conjunctiva in sterile water. This was mixed, and 1 drop was placed in known sterile solutions of fluorescein and merbromin (1:100). Cultures of these revealed organisms (Staphylococcus and Bacillus subtilis) in the aqueous solution and the merbromin. The fluorescein solution with 1:5000 thiomerosal did not culture organisms.

A typed culture of P. aeruginosa of human origin (ATCC 10137) was inoculated (10^9, 10^8 . . . . 10^1 organisms) in duplicate into nine vials of fluorescein containing 1:5000 thiomerosal and, similarly, into nine vials in duplicate of fluorescein containing 1:3333 thiomerosal and polymyxin B sulfate, 500 units per cubic centimeter. As controls, duplicate tubes of broth were similarly inoculated with each set. The vials were subcultured to broth (Tr.S.B.) daily for five days, and after this time the broth cultures were streaked onto blood agar plates. These plates were incubated 48 hours. All subcultures from solutions containing thiomerosal and thiomerosal with polymyxin B sulfate were negative. Broth controls were positive throughout the experiment, subcultures showing abundant growth of P. aeruginosa.

It is noted that 1:5000 thiomerosal was effective against this particular strain of P. aeruginosa. The possibility of a strain of P. aeruginosa resistant to 1:5000 thiomerosal and sensitive to polymyxin B sulfate exists.

The highest dilution of merbromin killing micro-organisms after a 10-minute period, but not after a 5-minute contact at 37 C is compared with that for thiomerosal (Birkhaug [14]) in the following tabulation.

Organism	Merbromin	Thiomerosal
Staphylococcus aureus . .	1:160	1:120,000
Hemolytic Streptococcus .	1:320	1:112,000
Pneumococcus Type I . .	1:240	1:64,000
Escherichia coli	1:180	1:32,000
Gonococcus	1:240	1:48,000
B. subtilis	1:300	1:24,000

B. Merbromin (a fluorescein derivative—dibromhydroxymercurifluorescein): As a stain it is used in a 1% or 2% aqueous solution. The 2% solution produces better stain-

ing and is less likely to become contaminated but causes slightly greater irritation. It may be used for surface staining, the denuded areas staining red. Its coloring effect is inferior to that of fluorescein, and multiple instillations are usually required to secure this effect. It is relatively nonirritating to tissue, but more patients complain of burning and irritation from it than from fluorescein. Excess stain irrigates freely from the conjunctival sac. Penetrability for living tissue is poor. The bactericidal activity of merbromin is greatly reduced by the presence of organic matter. The hydrogen ion concentration affects the solution, which becomes relatively impotent if buffered to pH of body fluids. Merbromin, instead of being bactericidal, as is commonly thought, is relatively unsafe, in our opinion. Sterility is difficult to maintain without an additional preservative, and the solution is irritating in strengths necessary to insure sterility. Merbromin is not recommended for routine use as a stain.

C. Rose bengal (certified): This may be prepared as is fluorescein, in a 1% or 2% aqueous solution with 1:5000 benzalkonium chloride or 1:5000 thiomerosal as a preservative. It may be phosphate-buffered if thiomerosal is utilized, as benzalkonium results in precipitation. A 1% aqueous solution in 1:5000 benzalkonium chloride is employed in our clinic. This stain is useful as a diagnostic aid in suspected cases of keratoconjunctivitis sicca and suspected exposure keratitis (lagophthalmos, exophthalmos, ectropion) with desiccation of the epithelium in the interpalpebral zone.

Schirmer [10] introduced rose bengal in 1% solution for revealing the conjunctival changes accompanying deficient tear formation. Sjögren [11] recommends surface staining with this solution in the diagnosis of keratoconjunctivitis sicca. Forster,[10] in an effort to find a more reliable test for indicating early cases of this condition, compared the dye test with the filter paper test of Schirmer. He found good correlation in strongly positive cases, but not in mild cases. Thirty of 98 eyes analyzed showed a mildly positive rose bengal test, and for 23 of these the Schirmer test done first was negative. O'Day [15] reports favorable results using 1% rose bengal in chlorobutanol (Chloretone) water as an aid in the diagnosis of keratoconjunctivitis and recommends it in diagnosing cases of chronic conjunctivitis.

The dye appears to stain selectively devitalized conjunctival and corneal epithelium. Small red dots appear on the conjunctiva and cornea wherever epithelium is desquamating. The diagnosis of keratoconjunctivitis is probable in suspected cases when groups of staining spots are noted on the conjunctiva or cornea. The bulbar conjunctiva and caruncle stain intensely in the interpalpebral area. This frequently is cosmetically disfiguring for several hours.

Irritation is more frequent with rose bengal than with fluorescein. It is usually proportional to the devitalized area of the cornea and conjunctiva and to the injection of the conjunctival vessels. We have not often found it necessary to use surface anesthesia prior to staining with rose bengal. If tetracaine is used and a positive test results, the test may be repeated later without use of tetracaine in order to rule out a false-positive result.

Copious irrigation is required to remove excess rose bengal, and discoloration of the skin around the patient's eye and of the examiner's fingers is more pronounced than with fluorescein.

II. *Quinone Imide Group* (basic chromophore dyes).—A. The thiazins (methylene blue, polychrome methylene blue, and azure II): Methylene blue for vital staining should be the U. S. P., zinc-free, certified dye, since an especially pure product is needed. On standing, all methylene blue solutions oxidize and contain varying amounts of the lower homologs, primarily azure A and azure B. These are more violet in color than methylene blue and are more selective in their staining properties. Methylene blue which contains large proportions of these oxidation products is called polychrome methylene blue. Azure II is a mixture of equal parts of

methylene blue and azure I (a mixture of azure A and azure B).

Each of these dyes has been reported as having been employed for vital staining. Because of their close relationship, and since oxidation occurs so readily, resulting in mixtures of the three in a staining solution, they will be discussed simultaneously. They appear about equally effective, the latter two being more selective in their staining properties but exhibiting less penetration than the purer methylene blue.

These staining solutions are utilized in vital staining of nerve tissue, for which a nontoxic basic dye is required. The corneal nerves stain well, appearing as delicate bluish filaments, and the end-plates of nerve filaments can be similarly stained. Conjunctival lymph channels stain poorly, and subconjunctival injection has been recommended to accomplish this. Some parenchymal cells of the normal cornea can be stained, but this is exhibited more readily in diseased conditions. Epithelial cells retain stain, especially in such pathologic conditions as corneal ulcers, erosions, and keratitis. Subepithelial tissue is stained readily, e.g., phlyctenula, pinguecula, and episcleritis nodule.

These dyes are employed in 0.5% aqueous solutions. Pure methylene blue in 1% aqueous solution is satisfactory but produces more irritation. To obtain vital staining, 2 drops of solutions should be instilled at least three times at five-minute intervals. Simkova [16] obtained best results in vital staining of the cornea with a 0.3% solution of polychrome methylene blue of pH 7.9. He repeated instillation of the drops 8 to 10 times at five-minute intervals and then examined the patient every 15 minutes for an hour. Valuable information was obtained in following up the degenerative and regenerative phases in corneal diseases and comparing this with results obtained on histological examination of silver-impregnated cornea. Penetration in vital staining is enhanced by the prior use of cocaine. The effect of these stains is increased by contrast staining with scarlet red or fluorescein.

Considerable irritation may result from these stains. Topical anesthesia is recommended to prevent this, since it enhances the penetration of the drug at the same time. The eye should be patched if discoloration or irritation is prolonged, and especially if cocaine has been used.

Although these dyes are fair bacteriostatics, they are not very effective as bactericides. Goodman and Gilman [17] report methylene blue as bactericidal against the Staphylococcus in a 1:1000 dilution, but the dyes are unsafe against most organisms in strengths used. Esch. coli survives 25 minutes in a 1:10 dilution. The addition of benzalkonium chloride 1:5000 insures better sterility, and its action as a wetting agent improves penetrability. If sterility is checked frequently, these solutions may be kept on hand, as the oxidation which occurs (instability) increases staining activity.

Methylene blue (basic dye) may be mixed with equal parts of eosin (acid dye) to form a neutral stain.

B. The Oxazins (Nile blue sulfate and brilliant cresyl blue): These two dyes are prepared in a 0.5% aqueous solution. Both have been utilized for vital staining of the conjunctiva and cornea. Gaellemaarts [2] employed Nile blue sulfate as a 0.4% solution. Irwin [18] (1927) found cresyl blue the most favorable dye available for investigating penetration into living cells. It appears to have an especial affinity for epithelial cells. Subepithelial staining is obtained, but we do not consider the effect produced to be superior to that produced by methylene blue.

Irritation is minimal with 0.5% solution. Cocaine increases penetration and diminishes irritation. Some discoloration of the skin around the eye occurs, but this washes off.

III. *Azo Group* (basic dyes).—Scarlet red oil solution (Sudan IV certified) is the only important dye in this group. Scarlet red is soluble in oil (olive oil).

Scarlet red, 0.5%, is of greatest value in contrast staining when used as a counterstain with the quinone imide dyes for vital staining. It makes the tear film appear red, in contrast with the subepithelial blue

staining of the others. It is more satisfactory than fluorescein for this purpose.

Knüsel [19] states that the normal conjunctiva does not stain with either scarlet red or methylene blue except for isolated epithelial cells and droplets in the secretion of the tears. He reports that whenever such staining appeared in the grossly unchanged conjunctiva, some infiltration was found at biopsy. The stained corneal cells appear brown in reflected light and dark red in transilluminated light. The conjunctival cells are brown or wine-red.

Natural Dyes

Carmine (alum lake certified) is the only natural dye to be utilized as a staining agent for the conjunctiva and cornea. It is soluble at a pH of over 8 with sodium carbonate or borate and may be made up in a 0.5% solution in Gifford buffer, pH 8.47. It is incompatible with benzalkonium chloride, precipitation resulting, but is compatible with thiomerosal. We have found no specific indications for its use. Surface defects do not stain, but subepithelial tissue stains brownish red.

SUMMARY AND CONCLUSIONS

Various dyes which have been utilized most frequently in ophthalmology for staining of the conjunctiva and cornea are discussed. The literature is reviewed, and an attempt at evaluation has been made at this clinic.

Three staining solutions would serve most ophthalmologists sufficiently well. Fluorescein is still the most satisfactory stain for general use if strict sterility is maintained. Preliminary evidence indicates that the utilization of polymyxin B sulfate in this staining solution offers additional protection against contamination by P. aeruginosa. Rose bengal is more valuable in diagnosing early cases of keratoconjunctivitis sicca. Methylene blue is adequate for vital staining of subepithelial tissues, and its effect is enhanced by prior use of cocaine and with a counterstain of fluorescein or scarlet red for contrast.

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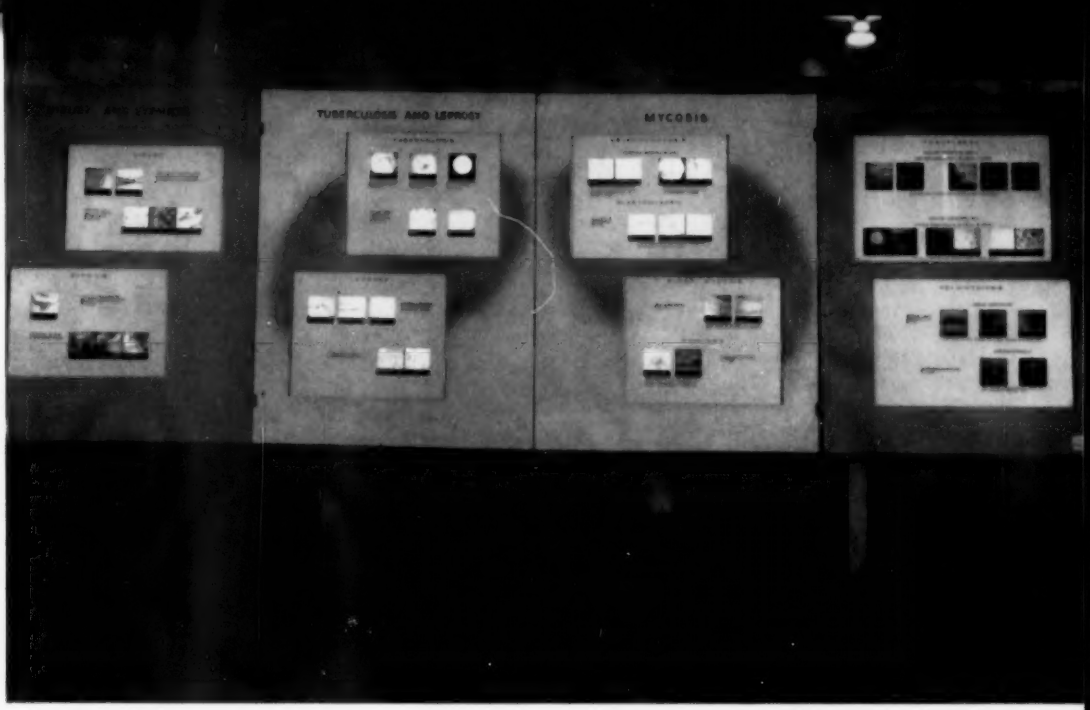


Scientific Exhibits

ORGANISMS IDENTIFIED IN INFLAMMATORY LESIONS OF EYES IN THE REGISTRY OF OPHTHALMIC PATHOLOGY

Armed Forces Institute of Pathology, Washington, D. C.

HELENOR CAMPBELL WILDER

WASHINGTON, D. C.

AND

MAJOR JOHN H. BICKERTON

MEDICAL CORPS, UNITED STATES AIR FORCE

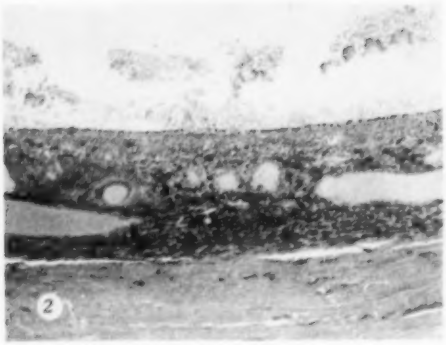
INTRANUCLEAR inclusion bodies resembling those of herpes simplex, Treponema pallidum, Mycobacterium tuberculosis, Mycobacterium leprae, Mucor, Candida, Toxoplasma, Cysticercus, and nematode larvae have been demonstrated in intraocular granulomatous lesions in the Registry of Ophthalmic Pathology at the Armed Forces Institute of Pathology, and examples of the lesions which they incite, with brief clinical histories and statistical data, are included in the exhibit.

Shown as a scientific exhibit of the Section on Ophthalmology at the 103rd Annual Meeting of the American Medical Association, San Francisco, June 21-25, 1954.

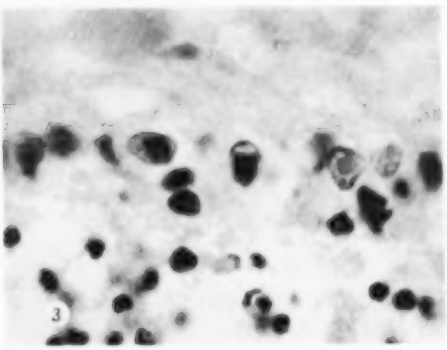
This exhibit was awarded first prize in the scientific exhibits at the Annual Meeting of the American Academy of Ophthalmology and Otolaryngology in Chicago, in 1953.

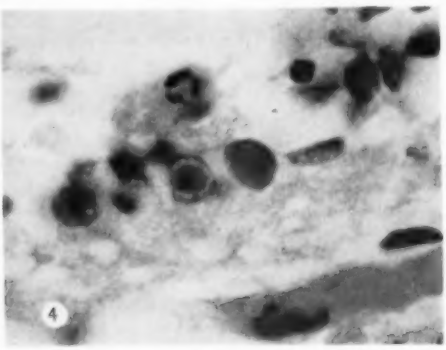
PANEL I—VIRUS? AND SYPHILIS

VIRUS?



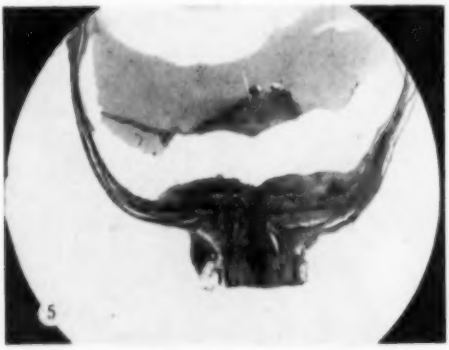
From a 59-year-old white woman with uveitis of two years' duration. No history of injury. Wassermann and tuberculin reactions negative.



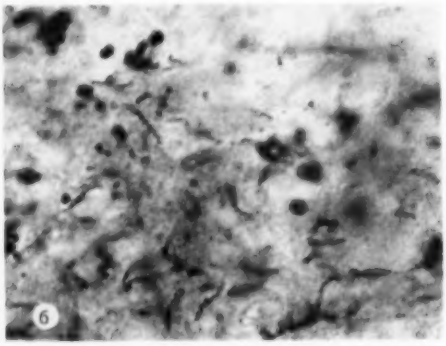


Intranuclear inclusion bodies demonstrated in one eye.

SYPHILIS



From a 39-year-old white man who had been treated for chancre. Wassermann negative at time of enucleation.



Treponema pallidum demonstrated in two eyes.

LEGENDS TO PANEL I

Fig. 1.—Diffuse granulomatous uveitis. A. F. I. P. 62192.

Fig. 2.—Granulomatous choroiditis; acute retinitis with necrosis. A. F. I. P. 62192.

Fig. 3.—Intranuclear inclusion bodies in retina. A. F. I. P. 62192.

Fig. 4.—Intranuclear inclusion bodies in retina. A. F. I. P. 62192.

Fig. 5.—Syphilitic chorioretinitis and optic neuritis. A. F. I. P. 114775.

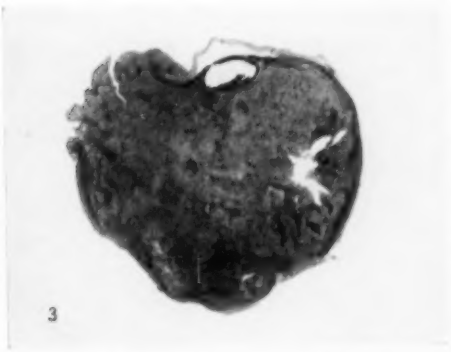
Fig. 6.—Treponema pallidum in chorioretinal lesion. Warthin-Starry stain. A. F. I. P. 114775.

PANEL II—TUBERCULOSIS AND LEPROSY

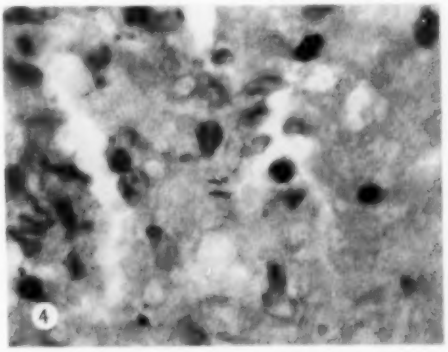
TUBERCULOSIS

From a 5-year-old Korean girl with tuberculous lymphadenitis.

From a 34-year-old white woman with pulmonary tuberculosis.



From a 17-year-old Negro girl with pulmonary tuberculosis.



Mycobacterium tuberculosis demonstrated in 10 eyes.

LEPROSY

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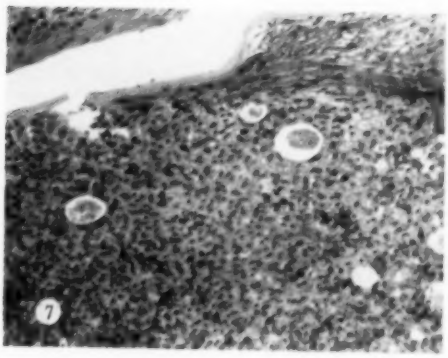
Eyes enucleated during life from adults with lepromatous leprosy.



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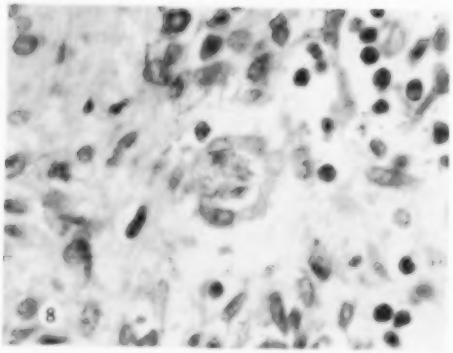

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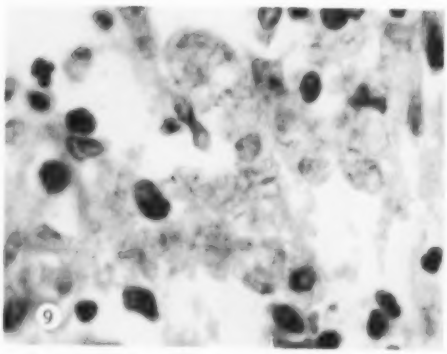

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Mycobacterium leprae demonstrated in 28 eyes.

LEGENDS TO PANEL II

Fig. 1.—Tuberculous iridocyclitis. A. F. I. P. 539890.

Fig. 2.—Tuberculous chorioretinitis and endophthalmitis. A. F. I. P. 185597.

Fig. 3.—Tuberculous panophthalmitis. A. F. I. P. 84599.

Fig. 4.—Mycobacterium tuberculosis in giant cell in chorioretinal lesion. Acid fuchsin. A. F. I. P. 185597.

Fig. 5.—Leprous iridocyclitis. A. F. I. P. 123164.

Fig. 6.—Lepra cell forming iris "pearl." A. F. I. P. 217657.

Fig. 7.—Lepra and epithelioid cells in ciliary body. A. F. I. P. 123164.

Fig. 8.—Mycobacterium leprae in giant (lepra) cell. Acid fuchsin. A. F. I. P. 217656.

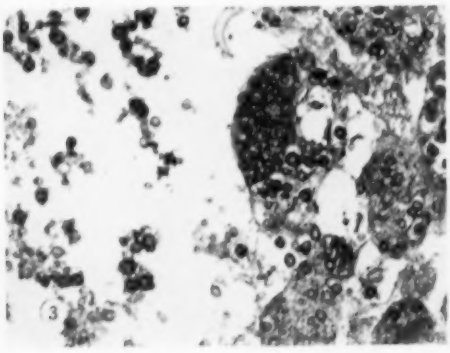
Fig. 9.—Mycobacterium leprae in epithelioid "foamy" cells. Acid fuchsin. A. F. I. P. 217656.

PANEL III—MYCOSIS

CRYPTOCOCCOSIS

From 29-year-old white man who died with malignant lymphoma. Cryptococcus infection of lung and choroid. Eye removed at autopsy.

Cryptococcus demonstrated in three eyes.

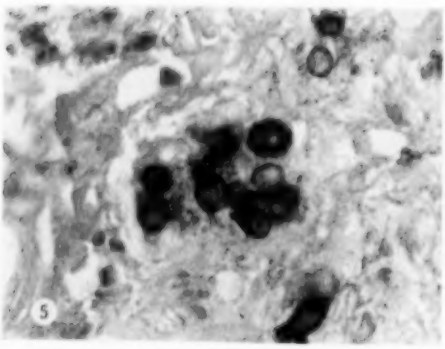


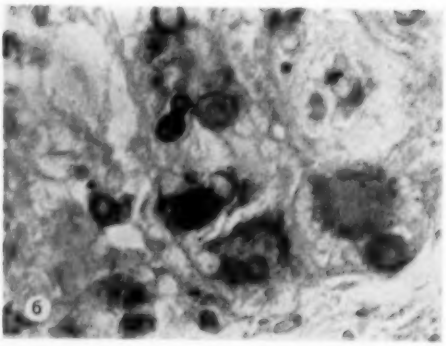
From 76-year-old white woman with ocular inflammation for four months. No other symptoms of disease.

PANEL III—MYCOSIS (Cont.)

BLASTOMYCOSIS

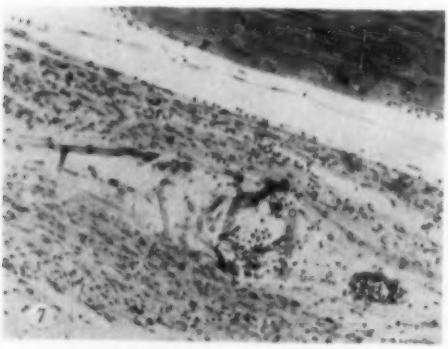
Blastomyces demonstrated in two eyes.

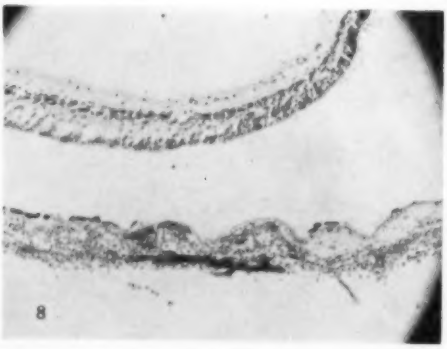




From 65-year-old white man who died with generalized blastomycosis. Eye removed at autopsy.

MUCOR MYCOSIS



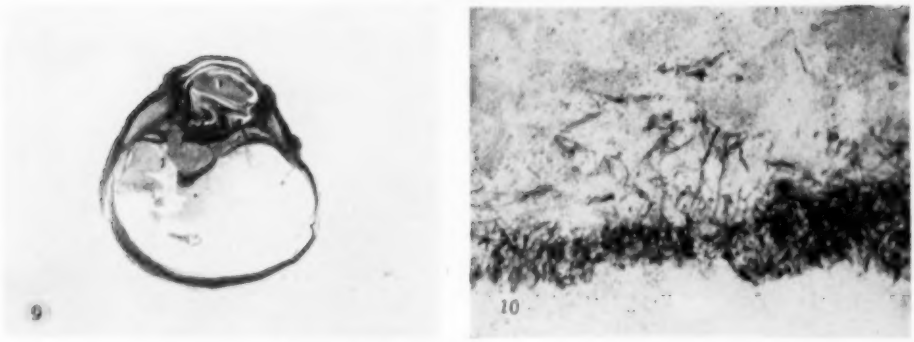


Mucor demonstrated in one eye.

From 73-year-old white man who died with Mucor meningitis. Eye removed at autopsy.

PANEL III—MYCOSIS (Cont.)

MONILIASIS



Candida demonstrated in one eye.

From 30-year-old white man who died with Candida meningitis. The clinical diagnosis has been tuberculosis. Eye removed at autopsy. Case reported by J. B. Miale, M.D.

LEGENDS TO PANEL III

Fig. 1.—Granulomatous lesion in choroid. A. F. I. P. 262406.

Fig. 2.—Acute cryptococcic chorioretinitis. A. F. I. P. 161194.

Fig. 3.—Cryptococcus in cells of retinal pigment epithelium and subretinal space. Mayer's mucicarmine. A. F. I. P. 161194.

Fig. 4.—Blastomycotic iridocyclitis. A. F. I. P. 87260.

Fig. 5.—Double-contour bodies of Blastomyces in giant cell. Gridley fungus stain. A. F. I. P. 87260.

Fig. 6.—Budding form of Blastomyces. Gridley fungus stain. A. F. I. P. 87260.

Fig. 7.—Mucor mycelia around circulus arteriosus major. A. F. I. P. 87679.

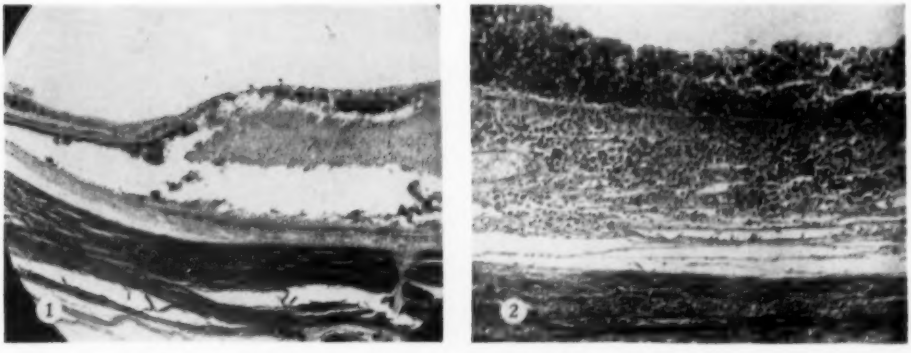
Fig. 8.—Mucor mycelia in choroid. A. F. I. P. 87679.

Fig. 9.—Candida abscess in vitreous. A. F. I. P. 238815.

Fig. 10.—Candida albicans in vitreous abscess. Bauer's leucofuchsin-methyl green. A. F. I. P. 238815.

PANEL IV—TOXOPLASMOSIS AND HELMINTHIASIS

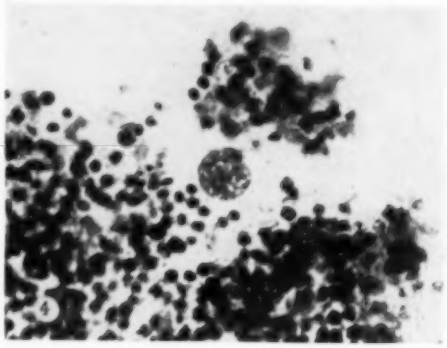
TOXOPLASMA



Toxoplasmic chorioretinitis, infantile

Toxoplasma demonstrated in the eyes of two infants

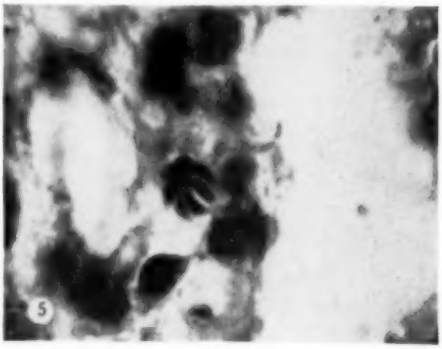
TOXOPLASMA

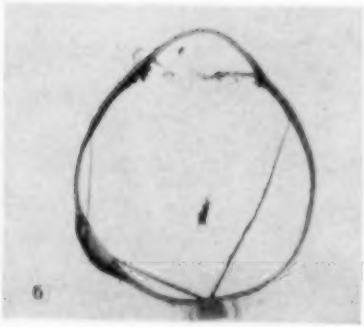


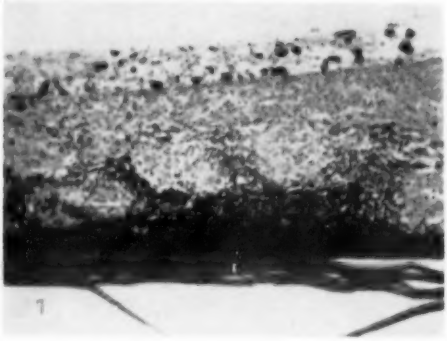
Toxoplasmic chorioretinitis, infantile

Toxoplasma demonstrated in the eyes of two infants (cont.)

Eyes enucleated at autopsy from infants dying with toxoplasmosis





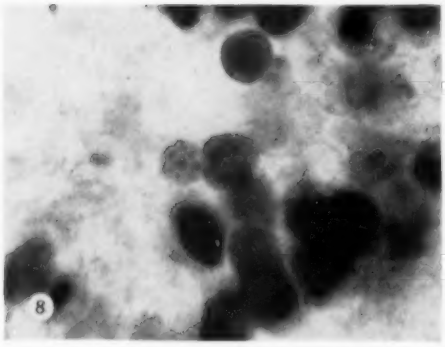


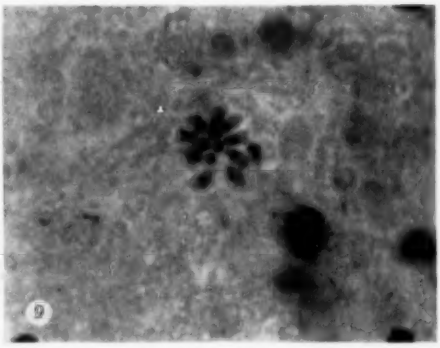
Toxoplasmic chorioretinitis, adult

Protozoa demonstrated in the eyes of 58 adults

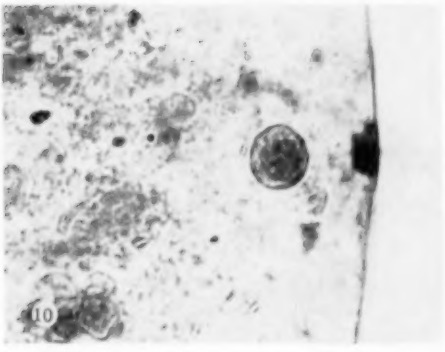
PANEL IV—TOXOPLASMOSIS AND HELMINTHIASIS (Cont.)

TOXOPLASMA





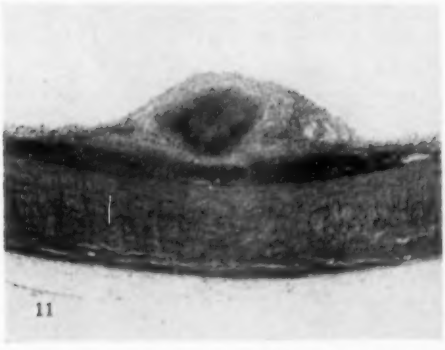
Toxoplasmic chorioretinitis, adult
Protozoa demonstrated in the eyes of 58 adults (cont.)

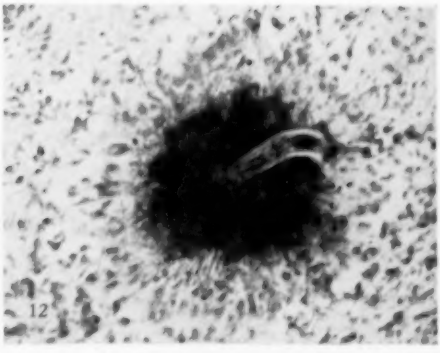


Eyes enucleated from adults without apparent systemic disease.

HELMINTHIASIS

NEMATODE ENDOPHTHALMITIS

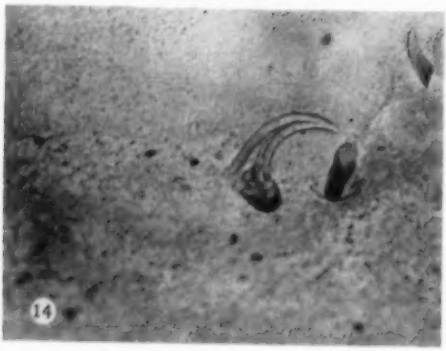




Nematode larvae demonstrated in 28 eyes.
Eyes enucleated from children with clinical diagnosis of retinoblastoma.

HELMINTHIASIS (Cont.)

CYSTICERCUS, INTRAOCULAR



Cysticercus demonstrated in two eyes

From 7-year-old Mexican boy with Taenia solium infection of intestinal tract.

LEGENDS TO PANEL IV

Fig. 1.—Toxoplasmic chorioretinitis with scleral involvement. A. F. I. P. 283307.

Fig. 2.—Toxoplasmic chorioretinitis with retinal necrosis. A. F. I. P. 197524.

Fig. 3.—Toxoplasmas in mononuclear cell in retina. A. F. I. P. 283307.

Fig. 4.—Toxoplasmic pseudocyst in retina. A. F. I. P. 283307.

Fig. 5.—Toxoplasmas in rosette formation in retina. A. F. I. P. 283307.

Fig. 6.—Toxoplasmic chorioretinitis. A. F. I. P. 211318.

Fig. 7.—Toxoplasmic granulomatous chorioretinitis with retinal necrosis. A. F. I. P. 171510.

Fig. 8.—Intracellular protozoa in retina. A. F. I. P. 482589.

Fig. 9.—Protozoa in rosette formation. A. F. I. P. 165904.

Fig. 10.—Necrotic cell or pseudocyst containing ill-defined protozoa in necrotic retina. A. F. I. P. 70313.

Fig. 11.—Eosinophilic abscess in chorioretinal lesion of nematode endophthalmitis. A. F. I. P. 132401.

Fig. 12.—Nematode larva in eosinophilic abscess surrounded by granulomatous reaction. A. F. I. P. 69623.

Fig. 13.—Cysticercus in vitreous. A. F. I. P. 198704.

Fig. 14.—Hooklet of Cysticercus. A. F. I. P. 198704.

Annual Reviews

LENS AND VITREOUS

JOHN M. McLEAN, M.D., New York

The year since the last review on this subject [1] has been marked more by the quantity of papers on the lens than by any great advance in knowledge. Approximately two-thirds of the contributions are about details of surgical technique, few of them new. Roughly a quarter of these concern experiences with intraocular acrylic lenses. The more fundamental reports deal mostly with the chemistry of the lens in relation to cataract formation and the mechanism of radiation cataract. Literature on the vitreous is rather sparse.

LENS

Ridley Lens.—The one subject which has drawn greatest attention is the intraocular implantation of a plastic lens at the end of cataract extraction. It does not seem worth while to review all of the small papers which report performance of the Ridley operation but contribute nothing else.

Ridley [2] himself reports his continued experience with the operation and his generally good results. He reviews the complications very completely and feels that the operation can be strongly recommended. In one instance he successfully performed a posterior discission of the capsule behind the implant to improve vision. A series of over 100 cases yielded final acuity of better than 6/9 in 66% and less than 6/60 in only 7%.

The results and opinions of others who have tried the operation vary. In this country, Reese [3] seems to have had the greatest experience, and his opinion is distinctly favorable. Of 29 cases of attempted implant after extracapsular extraction, he was unsuccessful in 2. One other patient developed a severe uveitis with glaucoma which required iridectomy, and one lens became partially dislocated and had to be removed. The overall visual results compare favorably with those after the classic extracapsular operation.

At the very same meeting where this report was given, Finlay and Romaine [4] rendered a more pessimistic opinion. Of 11 cases, attempts at insertion of the lens were abandoned in 2 because of vitreous loss. Two implants were removed later for severe uveitis and glaucoma. In one of these there was a possible sympathetic ophthalmia. Three cases developed occluded pupils. In one of these the lens was subsequently depressed in an attempt to open the occlusion. Four patients finally obtained 20/40 or better with additional correcting lenses, and two of them developed fusion with the normal fellow eye. All the authors' cases had prolonged postoperative iritis, and in most of them adequate fundus study was not possible.

After these two reports, the Cataract Panel of the American Academy of Ophthalmology and Otolaryngology rendered the opinion * that the Ridley operation is not yet sufficiently proved to justify general use in this country.

Both favorable and unfavorable opinions are reported from various countries. Arruga and Arruga [5] experienced three dislocations of the plastic lens—one at the time of operation, two about a year postoperatively. They were well tolerated in the vitreous, although one patient developed a transient glaucoma. These authors concluded that the ultimate results were good in spite of the postopera-

* Fry, W. E., in discussion on Reese [3] and Finlay and Romaine.[4]

tive reaction and slow elimination of pigment deposited on the face of the lens.

Malbran and Salleras [8] cite 15 cases with 3 dislocations after intracapsular extraction. Visual acuity was better than 0.5 in six cases, better than 0.1 in two, and less than 0.1 in four. One patient who had had a monocular cataract and exotropia developed acuity of 1.0 and third-degree fusion in 40 days.

McLean [7] reports final vision of 20/40 or better in 9 out of 17 cases and 20/100 to 20/60 in 4 more. He stresses as complications vitreous hemorrhage, pigment migration, and secondary glaucoma. Patwardhan [8] cites two good results with short follow-up.

Naim [9] reports four cases, with gross tilting of the lens in one. He feels that the operation will be very useful in Egypt, where glasses severely handicap the field workers.

Anton † implanted the plastic lens four times at cataract extraction and twice months afterward. In all six instances the results were satisfactory.

Other encouraging reports include those by Miller and Carton,[12] based on 12 cases, and Gupta,[13] who stressed the amount of pigment deposited on the lens.

Dolcet [14] is less encouraging, with poor results in all of seven cases. One lens was lost in the vitreous three months later; two had to be removed. Two pupils became occluded. One patient finally achieved 6/36 vision, and one died.

Jonkers [15] reported severe postoperative uveitis, which he ascribed to reaction of the iris to the plastic. Desvignes ‡ considered the operation unsafe.

Palazon,[18] Silvan Lopez,[19] and Guzman [20] all described suction cups to handle the lens in its insertion and centering. The last of them also commented on the advantage of the suction cup when removal of the lens became necessary.

Monocular Aphakia.—Other than the Ridley lens, methods for rehabilitating patients with monocular cataracts received little attention in the past year. Cowan [21] urged that a patient with one good eye be left alone. He felt that neither contact lenses nor any other device could restore useful binocular vision to patients with monocular aphakia. He claimed on theoretical grounds that fusion would be impossible.

Constantine and McLean,[22] however, were able to show that 25 of 30 patients with monocular aphakia were able to obtain fusion with contact lenses. They cite 60 to 62 patients with apparently satisfactory binocular vision. In seven patients with third-grade fusion aniseikonia measured from 4% to 10%.

Similarly, F. Ridley [23] studied 108 cases of unilateral aphakia; 43 were fitted with contact lenses. Eight of them had suppression and gave up the lenses, but 10 wore them all day and 25 for varying times. He felt that the interval between the development of the cataract and operation was more important than the period between operation and fitting of the lens.

It would seem, then, that contact lenses do offer a practical solution to the dilemma of monocular aphakia until adequate appraisal and refinement of the Ridley operation can be had. Certainly, potential danger to the eye is less when the plastic material is not intraocular.

Cataract Surgery

Much of the literature of the past year relates to surgery of the lens. The most complete discussion is to be found in the symposium of the American Academy of Ophthalmology and Otolaryngology. The late Daniel B. Kirby, who served as moderator, outlined [24] the indications for operation, the variations in technique with different surgeons, and the usual means of estimating prognosis. He advocated intracapsular extraction in all but young patients and stressed the need for flexibility of surgical methods to meet complicating situations.

Atkinson [25] discussed preparation of the patient, stressing asepsis and antimicrobial agents. He urged individualization of sedatives and analgesics and adequate local anes-

† References 10 and 11.

‡ References 16 and 17.

thesia and akinesia. Intravenous thiopental and intravenous curare were offered as alternatives.

Davis [26] stated his personal preference for knife sections but recognized the growing popularity of keratome-scissors techniques. Both are discussed in detail. He cautions against a knife that is too sharp. (This is a problem which has never plagued the reviewer.) Corneoscleral sutures are strongly urged, and various types are illustrated and described, including his own. Peripheral iridotomy is preferred, but peripheral iridectomy and complete iridectomy are described.

Fry [27] discussed extracapsular extraction, suggesting that it might be used for mature or hypermature lenses and in patients under 30 years of age. He also described extracapsular completion of operation when intracapsular extraction had been planned.

Vail [28] outlined the usual methods of intracapsular extraction with emphasis on the structure of the zonule.

McLean,[29] in discussing modifications in technique in the face of complications, suggested shallow, corneal incisions in eyes prone to bleed and deep, entirely scleral incisions for Fuchs's dystrophy. Full iridectomy was recommended in narrow-angle glaucoma, old uveitis with massive synechias, rigid pupils, and with deep scleral sections. Radial iridotomy was suggested when there is danger of a "hammock" pupil. Adrenocortical steroid therapy was recommended when a lens must be extracted in the presence of uveitis.

Clark [30] discussed complications at operation. When retrobulbar injection produces bleeding, the operation is abandoned, and he prefers other methods of anesthesia and akinesia when it is again undertaken. Free use of canthotomy is advocated for adequate exposure. Cautery is suggested to control oozing vessels. Means of eliminating pressure to prevent vitreous loss are discussed.

Chandler [31] advised good corneoscleral sutures to prevent flat anterior chamber and repair of wound leak when it occurs. Air injection and posterior sclerotomy may be used when no leak is found. He urged prompt repair of iris prolapse. Anterior chamber hemorrhage was treated expectantly unless increased pressure supervened, when paracentesis was advised. He considered corneal edema a dangerous complication and urged early efforts at freeing any vitreous from contact with the cornea. Pupillary block was reviewed and early relief by iris incision or excision advocated.

Finally, Guyton [32] described and illustrated the histopathological picture of many of the commoner complications.

Wound Closure.—A number of individual papers on wound closure have appeared without any major innovations. Vila-Coro [33] gives a long review of the subject, stressing the prophylactic value of good sutures. Dellaporta [34] publishes a similar, but shorter, review.

A few minor modifications have appeared. Stallard [35] has adopted a single "McLean-type" corneoscleral suture with a large knot on the corneal side so that the suture can be used to open the wound. Alvis [36] has also modified this style of preplaced suture by making it mattress in type.

Lo [37] and Shah [38] have each modified the Verhoeff track suture, while Krwawicz [39] has developed a continuous corneoscleral suture.

Golovine [40] has returned to the old technique of conjunctival bridge without sutures for operation on "unruly" African natives. His rationale is a trifle difficult to follow.

Dunnington and Regan,[41] in the Bedell Lecture, report their further studies on sutures and cataract wound healing. They found less tissue necrosis and less epithelial ingrowth along the suture track with plain or mildly chromicized surgical gut than with silk. Plain gut caused a polymorphonuclear reaction with early disintegration of the suture, but chromic gut stood up at least seven days. The wound appeared well healed in 14 days, but broader and more vascular than when silk was used. Their conclusion is that mild chromic gut is at least as satisfactory as silk.

Incision.—The polemic on relative merits of knife or keratome-scissors section seems

to be finally subsiding. Zolotnikova and Klikova [42] argue for knife section and tumbling delivery of the lens. Gill [43] and Llorca [44] favor use of keratome and scissors.

Williamson-Noble [46] and Sobhy [45] both extol the merits of corneal sections in front of glaucoma fistulas. Mitkova [47] prefers corneal incisions for soft cataract.

Technique.—A number of general accounts of surgical technique have appeared in the past year. LeWin,[48] Meesmann,[49] and Epstein [50] give general accounts, while Syed [51] outlines his own approach and Gillett [52] advises corneoscleral sutures and early ambulation for the osteopathic surgeon. Kirby [53] and Frell [54] review causes of failure and complications. Pettit [55] correlates the preoperative testing with perforated discs and red and white lights with postoperative visual results.

Meesmann and Lange [56] advise homatropine and glaucosan (epinephrine and histamine) for preoperative mydriasis, with intracameral pilocarpine and air at the completion of operation. They feel that this combination reduces the incidence of iris prolapse whether iridectomy is done or not. In their hands 1% physostigmine is inefficient. They believe that acetylcholine, while an effective miotic, is occasionally responsible for iris bleeding.

Pereira [51] urges inferior radial iridotomy in cases of previous iridocyclitis or while vitreous is lost.

Smith, Fansanella, Rosenthal, and Hoffman [58] warn against the indiscriminate use of cortisone or hydrocortisone after discission or extracapsular extraction. On the basis of two clinical cases, rabbit experiments, and the literature, they conclude that the dangers are as follows: slowing of absorption of cortex, possible precipitation of glaucoma, delayed wound healing, delay in absorption of hyphema, and decreased resistance to infection.

Clark,[59] in a discussion of the management of hereditary cataracts, advises needling in patients up to the age of 12, linear extraction from 12 to 25, and intracapsular or extracapsular extraction thereafter.

Kirby and Flagg [60] review the combination of curare and local anesthesia, with which their experience has been most satisfactory. O'Rourke and Starr [61] also favor curare and oxygen, while Jayle, Jaquenoud, and Haudiquet [62] outline the advantages of curare-induced general akinesia and ocular hypotony in their series of 101 operations.

Ali Shah and Hussain [63] came out for intracapsular extraction with the erisophake.

Instruments.—Most of the reports of new instruments represent minor modifications. Sakic [64] has put a small blunt hook on the other end of the Arruga lens expressor to retract the iris and facilitate grasping the lens above. Santalices Muniz [65] has modified the erisophake tip, while Caccamise [66] has designed a small "microerisophake." He describes the operation with this device but then reports that he breaks capsules in 15% of his cases and loses vitreous in 5%.

Friede [67] describes operation on soft cataracts in patients under 35 years with a 3-mm-bore, blunt, bent needle, through which lens material is sucked out by mouth suction. It is claimed that this technique is superior to discission or linear extraction, with less residual swollen lens material. The sucker is also suggested for removing cortex in older patients after extracapsular operation. Adler [68] has canalized one blade of a lens forceps so that continuous irrigations will maintain some anterior chamber during manipulations.

Flieringa [69] describes a stainless steel ring which is sutured to the sclera in eight places 5 mm. behind the cornea. The additional rigidity is meant to reduce the risk of vitreous loss. The ring may be removed at the end of operation or may be left in place and removed a week later. In one-eyed patients the recti are also severed at the start of the operation and reattached at the end. The author has used this device since 1946, with "satisfactory" results, but does not give any statistical documentation to justify complicating the operation in this way.

Complications.—Schirmer [70] cites one expulsive hemorrhage in 1230 operations in the

clinic at Wiesbaden. This accident occurred during a fit of coughing four hours after combined extracapsular extraction under general anesthesia in a 78-year-old arteriosclerotic patient. Nonnenmacher [71] may have established a record by having three expulsive hemorrhages on one day. One patient had arteriosclerosis and hypertension but two had no vascular disease. Nonnenmacher tries to explain his unusually bad luck on the basis of a changing weather front, which may have affected the patients' vegetative nervous systems.

LeGrand [72] studied 114 cases, with a 30% incidence of hyphema. Arterial hypertension, diabetes, iritis, and high myopia appeared to be contributing factors. Corneoscleral sutures, rutin, vitamin K, and ascorbic acid are advised.

Weekers and Delmarcelle [73] studied 30 cases in which the anterior chamber re-formation was delayed over four days, comparing them with 30 uneventful cases. They conclude that the cause was choroidal detachment and the result anterior peripheral synechias, producing glaucoma. They suggest better wound closure as the prophylaxis; isofluorophate (DFP), retrociliary diathermy, and cyclodialysis as the treatment. (The reviewer wonders, if better wound closure is the prevention, whether choroidal detachment should not have been classed as a result rather than a cause.)

Hesselberg [74] describes the two main complications of vitreous in the anterior chamber as secondary glaucoma and corneal opacification. In one of his cases of corneal clouding he was able to force the vitreous back by air injection, with resultant clearing.

Villaseca [75] describes as a "new syndrome" late vitreous hernia through the pupil, emptying of the anterior chamber, and detachment of the choroid from pull on the vitreous base. He advises avoiding mydriasis early after operation if the patient is up and about or coughing and also air in the anterior chamber at the end of operation. Orzalesi [76] advises posterior sclerotomy for flat anterior chamber.

Nichols [77] analyzes in some detail the late macular changes which followed cataract extraction in seven eyes of six patients. Two men and four women, in an age range of 65 to 86 years, all experienced late edema of the macula. The time of onset could be set at 58 to 197 days in four eyes; in the other three it was not definitely known. Four patients were arteriosclerotic. One had had vitreous loss at operation. There were no other operative complications. Three had final acuity of 6/6; the worst result was 6/18. Nichols questions whether the sequence of events was forward movement of the vitreous with posterior separation and macular edema or the reverse. Since his patients tended to be anxious and dependent, with vascular instability, he feels that vasomotor dysfunction of the posterior retina and choroid is the best explanation.

Laval and Collier,[78] noting a small hole in the hyaloid, with vitreous strands protruding to the anterior chamber, feel that it was probably the site of a slight lens adhesion.

Phacoanaphylaxis.—In studying lens allergy responses, Agarwal and Mehra [79] conclude that cysteine is the important amino acid fraction of lens protein. They advise avoiding extracapsular operation in patients who are intradermally sensitive to cysteine.

Müller,§ in comparing the clinical and histological features of phacogenic ophthalmitis, found accurate diagnosis difficult in the individual case. He studied two cases histologically, in each of which Morgagnian cataract had been accompanied by severe uveitis and glaucoma. Each had had extracapsular operation in the fellow eye—one 11 years before; one, 13. The aphakic eyes were quiet. The enucleated eyes showed macrophage activity around the hypermature lenses and lens debris blocking the angles. There were spontaneous defects in the capsules. He concludes that, although it is difficult to decide whether the effect is primarily a toxic one from degenerated lens substance or is secondary to phacoanaphylaxis, cataracts should not be allowed to become hyperma-

§ References 80 and 81.

ture. He advises total lens extraction and cortisone. It is interesting to compare his attitude with Cowan's advice, cited before.[21]

Glaucoma from Lens Changes.—Dvorak-Theobald [82] studied three eyes enucleated with the clinical diagnosis of wide-angle glaucoma and capsular exfoliation. She found the histological picture different from the classic description. There were amorphous deposits on the capsule and in the angle without any true exfoliation. She feels that this may occur in any patient but that true exfoliation occurs only after infrared exposure. Thus, true exfoliation results in cataract; pseudoexfoliation, in glaucoma.

Wilson [83] studied 27 cases clinically. He found that 8 had exfoliation without increased pressure, while 14 had increased pressure and field defects. He felt that exfoliation was not a primary cause of glaucoma, nor glaucoma of exfoliation. He suggests the term "glaucoma senilis" and advocates intracapsular operation.

Stepanik [84] reports a case of glaucoma capsulare treated successfully by goniotomy.

Lens Changes from Glaucoma.—Emphasis is again made on lens changes after fistulizing operations. Legrand [85] stresses their frequency, and Hobbs and Smith [86] cite four cases of capsule adherent to trephine opening with cataract formation in three. They urge slow decompression.

Krol [87] describes delicate lens changes following attacks of acute glaucoma. They do not always occur and never are seen in chronic glaucoma without acute episodes. He feels they are the result of disturbance in intraocular tissue balance.

Nonay [88] records 69 cases of primary glaucoma with cataract. Many had had previous glaucoma operations. He did a combined intracapsular operation in 50 and a combined extracapsular operation in 19. Good vision was obtained in some 40%.

Miscellaneous Changes.—Reese and Wadsworth [89] describe cystoid changes along the anterior and posterior borders of the adult nucleus in 10% of normal eyes in patients over 40. Their nature is not identified. There was nothing unusual in the blood chemistry, and they were not related to the usual senile cortical or nuclear cataract.

Winkler and Cason [90] studied 11 infections in 1653 cataract extractions. Ten were caused by Gram-negative bacilli of the enteric group. Nine resulted in enucleation.

Cataract associated with skin disease received attention from Dubois-Poulsen and François,[91] who found rapidly developing cataracts in three young patients with childhood eczema, and Hertzberg,[92] who found a 10% incidence of atopic cataract in disseminated neurodermatitis.

Nutt[93] contrasts modern cataract surgery in diabetics with that of the preinsulin days and finds the incidence of hyphema and iritis markedly reduced. He advises ascorbic acid empirically to reduce the risk of hemorrhage, and intracapsular extraction to minimize iritis.

Studying the statistics of Pakistan, Refatullah [94] finds true diabetic cataract very rare, only 3 in 7500 cases. Cataract is common in Pakistan, with 60,000 blinded by it each year and only 20,000 operated on. Couching is still common, with 4000 or 5000 such operations done each year. Ninety per cent of the lost eyes are due to couching. Eighty per cent of all eye operations in Pakistan are for cataract.

Linn and co-workers [95] studied 21 patients with postoperative mental aberrations. Twenty were disturbed by binocular bandaging; 18 had evidence of organic brain damage, suggesting a correlation between cataract and general senility.

Dienstbier [96] reports successful intracapsular cataract extraction in an eye quiet for six years after penicillin treatment for sympathetic ophthalmia.

Arruga || reports a case of anterior dislocation of Soemmerring's ring and reviews the literature.

Corrado [99] and Velhagen [100] discuss in a general way the management of secondary membranes.

|| References 97 and 98.

Experimental Study

Research on the lens follows much the same pattern as in recent years. Cataracts from irradiation and abnormal carbohydrate metabolism have received much attention, with protein chemistry and enzymatic action also under scrutiny.

Radiation Cataract.—Further studies on the mechanism of lens injury by irradiation of selected areas were carried out by Puntenney and Schoch.[101] Rabbit eyes were exposed to 4000 r with lead shields confining the dose to lens or ciliary body or whole eye. With this system cataracts were produced respectively in 17%, 100%, and 100% of the eyes. The authors feel that the effect on the ciliary body is important and requires more study. It is interesting to compare this with the previous work by similar methods cited in last year's review.[1]

Cogan[102] reviews lesions from radiant energy, outlining the necessity of absorption of ionizing radiation for cataract formation. There must be sufficient to damage lens epithelium whether infrared, x-ray, gamma, high-voltage beta, or neutron. With Dreisler,[103] he studied 40 patients who had had radiation about the eye for from 1 to 13 years. Four with over 800 r developed cataract. One of 3 with 600 r developed a cataract in two and a half years, while 33 with 487 r or less did not develop cataractous changes. Thus the human threshold dose for cataract in this time period seems to be about 600 r. With Donaldson, Goff, and Graves,[104] he studied the effect of single doses on rabbit lenses. All the animals were observed for a year and a half, or about one-third their normal life span. The amount of radiation required to produce detectable lens changes was less than had previously been thought (250 r for x-rays and 1.0×10^9 neutrons/sq. cm.). Granular dots around the posterior suture line were seen after 75 r or 1.0×10^7 neutrons/sq. cm. This low-dosage effect, however, was minimal and in some instances regressed. It took about 500 r of x-rays of 1.2-mev energy and about 8.4×10^9 for neutrons of 14-mev energy and 3.2×10^{10} for neutrons of degraded fission energy to produce cataracts dense enough for serious visual impairment. Their onset was about 100 days after exposure. Calculations of energy release showed that with equivalent tissue ionization neutrons were more cataractogenic than x-rays.

Von Sallmann, Munoz, and Drungis[105] treated rabbit eyes over the limbus with 2,000, 5,000, 10,000, and 20,000 rep from a Sr^{90} applicator. Cytological examination showed damage to the nuclei of the germinative zone in the sector nearest the irradiated limbus only. Extent of injury varied with dosage. When 5000 rep or more was used, five-sixths of the rabbits developed lens opacities 10 weeks after exposure, although the cornea in direct contact was not effected. X-rays with 100, 500, and 1500 r produced similar changes. Caution is advised in human treatment with beta applicators near the limbus.

Moses, Linn, and Allen[106] used single exposure to a collimated beam of 6- to 8-mev neutrons of 60 to 150 N without producing cataract in dogs. The animals were followed two years. Exposure to 810 to 900 N produced cataract in 60%-75% of the adult dog eyes, while 1500 N gave necrosis of the globe and orbit in three to four months.

Pirie, van Heyningen, and Boag[107] studied unilateral radiation cataracts in rabbits, using the normal fellow eye as a control. They found a decrease in reduced glutathione before visible change in the lens. Glutathione-reductase activity was reduced, but other enzyme systems were unaffected.

A similar field is the study of ultrasonic lens damage. Lavine and Langenstrass[108] produce cataracts in excised eyes by ultrasonic vibrations. Schwab[109] treated isolated pig eyes with ultrasonic waves of varying intensity and time. Frequency of 1000 KH_s and intensity of 4.0 watts/sq. cm. yield vacuoles and white opacities in the cortex. Increasing the frequency to 5000 KH_s produced central opacities in seconds and total cataract in one minute from thermal action.

Carbohydrate Cataract.—The lens changes of abnormal carbohydrate metabolism con-

tinue to attract investigators. Inoe,[110] working with alloxan diabetes and dithizone, found that the combined administration of ascorbic acid, riboflavin, and potassium chloride impedes the development of experimental cataract and tends to reduce opacities already present.

Butterini, Grignolo, and Baronchelli [111] used 50 to 100 mg/kg. diphenylthiocarbazone in rabbits to produce widespread metabolic disturbances similar to alloxan diabetes. They produced lens changes similar to the cataract of alloxan diabetes and retinal degeneration similar to iodine poisoning.

Babel,[112] in a very lengthy discussion, compares the cataract of alloxan diabetes in rats and rabbits with toxic cataracts in humans.

Johnson [113] reviewed the literature on cataracts in galactosemia, pointing out that 12 of the 13 cases reported since 1945 showed lens changes. The sole exception was treated by the age of 2 weeks. He adds three cases of his own. He is unable to correlate patient age and type of lens change but notes that withdrawal of milk before the age of 3 months is necessary to prevent irreversible opacities. McAuley,[114] in reporting two cases, suggests an osmotic factor.

Hormann [115] studied cataracts in rats on diets high in lactose or galactose. He kept albino rabbits on a 70% carbohydrate diet. If alpha or beta lactose was fed, they became emaciated and died. The addition of 5-10 mg. of cocarboxylase every four days kept them alive and prevented lens changes. Thirty per cent alpha lactose and 40% oats produced lens opacities, reduced by cocarboxylase. The inference is that an excess of galactose interferes with carbohydrate breakdown, with accumulation of abnormal metabolites in the lens, increased permeability, and the entry of calcium.

Harris, Hauschildt, and Nordquist,[116] continuing their interesting in vitro technique of reversible cation shift with refrigeration and incubation recovery, studied the role of glucose in cation transport across the lens capsule. Glucose was found essential and could not be replaced by sucrose or l-glutamic acid. A high glucose concentration (500 mg. per 100 cc.) reduced the degree of cation recovery. This deleterious effect was nullified by adenosinetriphosphate (ATP), although the latter agent was toxic when added to normal glucose concentrations. Insulin had no effect. These results suggest that a high glucose level itself may be the responsible factor in diabetic cataract. Studying oxygen and acid,[117] they found a difference in cation exchange under aerobic and anaerobic conditions which could not be accounted for by the cation content of the epithelium alone. Thus it is suggested that the epithelium contributes energy for the whole lens, and that cations are actively transferred across the capsule. Glutamic acid failed to protect against high concentrations of ATP.

A study of the polysaccharide content of bovine lens capsule was made by Dische and Borenfreund.[118] They found a 3:2 ratio of galactose and glucose combined with the protein of the capsule to make up about 10% of the total capsule protein. The concentration is greatest at the center and falls off markedly toward the equator. What this has to do with carbohydrate cataracts is a matter for speculation.

Miscellaneous.—Leinfelder [119] tried to delineate further the role of respiration and glycolysis in lens metabolism. He felt that auto-oxidation of ascorbic acid and glutathione could be responsible for much of the oxygen uptake previously considered respiration. The presence of cytochrome oxidase suggests an active respiratory system in the epithelium. Low aqueous humor oxygen content and inconclusive results of carbon monoxide inhibition indicate that only a small part is respiratory. Lactic acid in the aqueous and the nonenzymatic uptake of oxygen are evidence of primary glycolysis. The metabolic activity of the epithelium may be related to movement of water. With Schwartz and Danes,[120] he undertook to study this last phase further and show that the maintenance of constant lens hydration requires energy. The energy necessary to shift water in or out of the lens is derived from metabolic activity and may be affected by metabolic poisons or anoxia. Nordmann, Mandel, and

Izraelewicz [121] differ somewhat from previous conclusions of Leinfelder. Boiled calf lenses consume more oxygen than fresh lenses. Fresh lenses consume glucose and produce lactic acid. They feel it is wrong to deny enzymatic lens respiration on the basis of boiling experiments, for the auto-oxidation may be dependent on protein denaturation by boiling. Boiled liver, similarly, consumes more oxygen, but enzymatic action in liver is well accepted.

Pare [122] indicated that lens capsule, epithelium, and superficial stroma function as a membrane with selective permeability and that most cataracts are caused by permeability changes. He cites diabetic, tetany, naphthalene, thallium, and radiation cataracts as caused by permeability disturbances and suggests cysteine for treatment. Studies of metallic precipitates show silver and copper to be deposited where there is relatively little enzyme concentration, while the concentration of iron corresponds to the cytochrome-oxidase system. Alajamo and Ambrosio,[123] using Pau's methods in the rabbit, studied capsule permeability in cataract associated with retinal detachment and found no change. They felt some other factor must be responsible.

Lyda and Waugh [124] studied the capsule from a very different approach. They made posterior needle cuts of the rabbit lens capsule and sectioned the eyes at different time intervals. As the inflammatory cell response from ciliary body and retinal vessels diminished, the capsule began to heal by epithelial proliferation. They compared their rabbit sections to human eyes in which after various types of trauma, without infection, there was posterior proliferation of lens epithelium. This reaction seemed to be inhibited by the presence of leucocytes.

Irregular increase in aqueous potassium content was found by Sugawara [125] in iridocyclitis, glaucoma, and cataract. Special staining indicated a decrease and a distributional change in lens potassium with cataract development. Martinet, Baron, and Guillon [126] studied sodium and potassium concentration in the lens and whole body of fetal rats made cataractous by feeding thyroxine to the mothers during pregnancy. Lens sodium concentration was increased as compared with controls, but potassium remained unchanged. There was no change in total body sodium or potassium.

In studying the lens metabolism of ascorbic acid, Soya [127] found its synthesis from diketogulonic acid impeded by naphthalene and calcium but accelerated by manganese. In line with this, Ichihara [128] found that ascorbic acid protects against naphthalene cataract.

Yamada [129] gave scorbutic guinea pigs 250 mg. of intraperitoneal sodium butyrate a day. After seven days he added 500-mg. daily oral doses of tyrosine. Cataracts formed five days later, with considerable benzoquinone acetic acid in the urine. When sodium butyrate was omitted, neither the cataracts nor the urinary changes occurred.

Gemolotto [130] made electrophoretic protein determinations on lenses of eyes subject to sodium iodate chorioretinal degeneration and found variations similar to those in cataracts. Electrophoretic studies of water-soluble proteins were also made by François, Wieme, Rabaey, and Neetens.[131] They found three separable fractions in ox, horse, pig, and rabbit lenses, four in the lenses of man and monkey. They could not find a major difference between human cataracts and normal lenses of the same age.

Dorello [132] was unable to find an appreciable change in glutathione content of the lens with large doses of corticotropin, DCA, cortisone, or formalin. Brolin [133] looked for changes in fluorescence of the lens in tetany cataracts but could not demonstrate any.

Auricchio and Ambrosio,[134] in the fourth of their series of studies on complicated cataract, found a decrease in pyruvic and lactic acid in the aqueous in the presence of iridocyclitis. These studies were done in rabbits, and their lenses had corresponding increases in these products.

Bonavolonta [135] studied oxygen in aqueous of normal eyes and those in which cataracts had been produced by lens massage. He found aqueous oxygen approximately doubled in the presence of this type of cataract,

indicating reduction of lens respiration. The same author [136] confined guinea pigs and rabbits in closed spaces to produce asphyxia and produced transient lens opacities in 25%. Aqueous carbon dioxide was increased; oxygen and chlorides were decreased. The animals returned to normal 30 minutes after release.

Haft [137] describes four patients with idiopathic hypoparathyroidism who developed cataract while under control with calcium and dihydrotachysterol. Centanni [138] found that subconjunctival hyaluronidase hastened the absorption of lens material in the rabbit. Sapuppo [139] found no effect from fish lens extract on experimental naphthalene cataract.

Gleserov [140] found cataracts in munitions workers in trinitrotoluene plants. The extracted lenses assayed around 0.001γ TNT.

Hallerman and Meisner [141] report a case of lens invasion by tumor cells from a malignant melanoma of the ciliary body. They dispute Ida Mann's remarks about tumor immunity of the lens. (It is the reviewer's opinion that these findings are not in conflict with Mann's views, for she was discussing tumor formation from the lens rather than invasion of a nearby neoplasm.)

VITREOUS

Vitreous Replacement.—Removal and replacement of abnormal vitreous is still a clinical problem. Binder,[142] working on rabbits, found simultaneous aspiration of vitreous and replacement by saline solution through a double-barreled needle safer than successive steps through a single needle. Paufique and Moreau [143] report interesting preliminary studies in which they used a dehydrated powder of lyophilized vitreous to replace vitreous lost at operation or injury or withdrawn because of opacity. Deutch [144] reports a single clinical case in which vitreous hemorrhage had been present five years. After 1.5 cc. replacement with cerebrospinal fluid, vision improved from light perception to 20/40.

Examination.—Hruby [145] reviews biomicroscopy of the vitreous and stresses the need for full mydriasis in the patient and dark adaptation of the examiner. He finds anterior detachment rare but posterior detachment common and important with separation of the hyaloid from disc and retina. The vitreous may retract to its base. This is seen in myopia, senility, trauma, uveitis, and detached retina with tears. Vitreous adhesion to the operculum of a tear can often be identified in detachment of the retina.

Schepens,[146] similarly, reviews clinical examination of the vitreous and in the main agrees with Hruby. However, he finds that vitreous liquefaction, or syneresis, may sometimes start centrally, leaving a resistant layer of formed vitreous around the periphery. He also finds that vitreous membranes are not always hyaloid, but that membranes formed at the limits of areas of syneresis may be mistaken for hyaloid and detachment, when a "pseudodetachment" exists.

Habachy, in two papers,¶ discusses vitreous opacities and tries to connect them with abnormalities of liver, kidney, endocrines, vegetative nervous system, acid-base balance, and vitamin intake. His recommendations for treatment are along similar lines.

Roveda [149] collected 43 cases of cyst of the vitreous from the literature and added 1 of his own. He notes their frequent association with retinitis pigmentosa.

Structure.—Belazs [150] divided fresh cattle vitreous into anterior and posterior parts and then separated the central and cortical portions of the posterior samples. These latter two portions were studied for ultraviolet absorption, in the fresh state, and dialyzed to remove low molecular weight components. Both central and cortical samples in the undialyzed state showed absorption bands between 200 and 300 mμ. One with a maximum at 265 mμ is attributed to ascorbic acid. After dialysis an absorption maximum at 277 mμ is caused by proteins. Proteins of the globulin type with relatively high tryptophan content were present in greatest quantity. The albumin-globulin ratio was estimated spectrophotometrically as 0.46.

The same investigator, with Varga,[151] studied vitreous by ultracentrifugation and

¶ References 147 and 148.

electrophoresis. A hyaluronic acid and a protein component were identified by means of treatment with specific enzymes and by addition of reference substances.

Shaffer [152] studied the possible factor that vitreous detachment may play in "malignant" glaucoma. In aphakic eyes or eyes with subluxated lenses, vitreous can cause blockage of the forward flow of aqueous. Posterior synechias to the vitreous face are known to cause such a block, which may be corrected by peripheral iridectomy or prevented by early postoperative mobility of the pupil. In malignant glaucoma not responding to intracapsular lens extraction, it is suggested that vitreous, iris, and ciliary body are so adherent that aqueous flows behind the detached vitreous and forces the structures anteriorly. The suggested treatment is incision through the detached vitreous to restore a channel for posteroanterior aqueous flow.

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News and Comment

ANNOUNCEMENTS

Association for Research in Ophthalmology Eastern Section.—The 1955 meeting of the Eastern Section of the Association will be held in Boston on April 25. It has been scheduled in conjunction with the annual meeting of the Massachusetts Eye and Ear Infirmary Alumni, on April 26, and the regular monthly meeting of the New England Ophthalmological Society, on April 27. All those interested are invited to attend the scientific sessions of these meetings.

The Association for Research in Ophthalmology is an open society. Membership in the Association is not a prerequisite for acceptance of a paper by the Program Committee.

Titles and abstracts are now being received. It is hoped that time of presentation can be limited to 10 minutes for each paper in order to save time for discussion. Abstract forms may be sent to the local chairman, Henry F. Allen, M.D., 200 Beacon St., Boston 16, Mass.

American Orthoptic Council Examination for Technicians.—The annual examination of orthoptic technicians by the American Orthoptic Council will be conducted in August and October, 1955.

The written examination will be nonassembled and will take place on Thursday, Aug. 25, in certain assigned offices and will be proctored by designated ophthalmologists.

The oral and practical examinations will be on Saturday, Oct. 8, in Chicago, just preceding the meeting of the American Academy of Ophthalmology and Otolaryngology.

Application for examination will be received by the office of the Secretary of the American Orthoptic Council, Dr. Frank D. Costenbader, 1605 22d St., N.W., Washington 8, D. C., and must be accompanied by the examination fee of $30.00. Applications will not be accepted after July 1, 1955.

Society Transactions

EDITED BY DR. W. L. BENEDICT

NEW YORK ACADEMY OF MEDICINE, SECTION OF OPHTHALMOLOGY

John M. McLean, M.D., Chairman

Joseph Laval, M.D., Secretary

Regular Meeting, Nov. 15, 1954

Closure of Orbital Defect After Exenteration for Carcinoma. DR. HERBERT CONWAY.

In advanced cases of carcinoma of the eyelid with invasion of the globe or the orbit, the accepted method of treatment is complete and radical excision of the upper and lower eyelids, together with complete exenteration. This treatment results in a very noticeable facial defect, which amounts to a major deformity of appearance. There are three methods of handling this problem. The first consists in the lining of the bony orbit with a thick-split graft of skin and the substitution of a prosthesis which embodies an artificial eye. An example is that of a patient aged 64 (New York Hospital Case #237954) who had an epidermoid carcinoma of the eyelids in the region of the inner canthus. The lesion recurred after therapy by electrodesiccation three years, and again one year, before the patient underwent radical surgery. At operation, performed at the New York Hospital in 1939, incision was made around the upper and lower eyelids, and the lesion, together with a good cutaneous margin, the globe, and all the tissues of the orbit, was excised en bloc. The tumor had invaded the eyeball. The periosteum of the orbital bones was removed with the specimen. A thick-split graft of skin was cut from the thigh and inserted into the bony orbit in such a way that it was in continuous contact with the bone and with the margins of the wound at all points. Bismuth tribromophenate (Xeroform) gauze was placed over the graft, and a mass of cotton waste was used to effect pressure on the lining of the orbital cavity. The graft healed, and the patient was fitted with a latex prosthesis colored to match the skin of the face. The prosthesis incorporated a glass eye. Rubber cement was used to attach the prosthesis to the skin of the face. The appearance of the patient was very good. However, discoloration of the prosthesis occurred with such frequency that it was necessary to provide the patient with a new prosthesis at three-month intervals. In the 15 years since 1939 some improvements have been made in prostheses. The vinyl prosthesis holds the color longer than does the latex prosthesis. However, this method leaves something to be desired in that the patient is dependent on this external crutch in order to pursue daily routine. This patient was followed for 13 years, during which period there was no recurrence of tumor. She died of heart disease. This type of management of the orbital defect after exenteration was popular in the years before World War II.

In the period during and after World War II, a great deal of experience was obtained with orbital defects as a result of war injuries. The popular method of closure was by the pedicled flap. An example of this type of closure is the case of a 54-year-old man (Veterans Administration Hospital Case #118466), whose basal-cell carcinoma of the eyelids recurred after radon therapy applied two years earlier. X-rays taken in 1948 showed destruction of bone due to the invasive growth of the carcinoma. In 1948 radical excision of the carcinoma was done through a Weber-Ferguson excision, sacrificing all of the soft tissues of the orbit, the malar bone, and the lining of the antrum. The defect was closed with a temporal flap of skin and soft tissue, which had been prepared by lining it with a thick-split graft of skin. This leaves a bland appearance, which is not attractive. Still, four years later, this patient showed no evidence of carcinoma, and he gets along very well through the use of spectacles with a darkened lens on the affected side.

Perhaps more ideal as a method of management of the orbital defect after exenteration is the technique employed in the third case to which I wish to refer. This is the case of a man aged 73 (New York Hospital Case #221323), who stated that a cyst was removed from his right upper eyelid in 1950. In September, 1953, he noticed that the right eye began to close. The lesion proved to be a basal-cell carcinoma, starting at the outer canthus and extending through the orbital tissue to the retro-ocular region. Here, again, the surgery was carried out by an exenteration and radical excision, but the defect was closed in a different way. The soft tissues were elevated from the forehead and relaxed by the use of horizontal incisions, which were carried through the periosteum and the fibers of the frontalis muscle, but not through the skin. Similarly, the soft tissues were separated from the anterior aspect of the maxilla, using the periosteal elevator. Mobilization of the soft tissues of the upper and lower portions of the wound was effected by this technique, so that the defect could be closed by linear suture, in horizontal direction. The relaxation of the upper flap by the use of the hidden relaxation incisions is the effective step in this technique. This method recognizes the fact that dead space, provided by the cavity of the orbit, must eventually fill with serum and blood. The effect of such a concentration of fluid is fibrosis, which results in a dimpled surface. The remnant of the eyebrow is dislocated downward. However, this technique is considered preferable to those described above, since the patient is not dependent on an external crutch for his appearance and the reconstruction can be accomplished at the time of the excision.

Intermittent Exotropia. Dr. Abraham Schlossman and S. Arthur Boruchoff, M.D., Boston.

This paper was based upon a study of all cases of strabismus seen in the Department of Motor Anomalies of the New York Eye and Ear Infirmary from 1951 through 1953. There were 1431 patients, of whom 1107 had esotropia and 324 had exotropia. The great confusion in terminology relating to exotropia was discussed, and several examples were given. The following classification for exotropia was recommended:

I. Intermittent exotropia

II. Constant alternating exotropia

III. Constant uniocular exotropia

Intermittent exotropia constituted 85% of all cases of exotropia. The term refers to those cases which at times show manifest divergent strabismus and at times exophoria or orthophoria, on the basis of the temporal and spatial measurement of the squint. Exophoria-exotropia is that condition in which, for any given distance of the object of fixation, the patient will at times show an exotropia and at times an exophoria of approximately similar degrees.

Intermittent exotropia was further considered on the basis of whether the patient has an exophoria, orthophoria, intermittent exotropia, or constant exotropia for near or for distance or for both. The characteristics of the five most important subgroups were discussed.

The incidence of myopia, emmetropia, and hyperopia paralleled that of the general population. Myopes constituted only 22.8% of the cases. Only 5% of patients with intermittent exotropia were benefited by the correction of myopia, and here the effect was mainly on the measurement for near.

In intermittent exotropia it appeared that fusion in the primary position is more apt to be "all or none" for distance fixation than for near; if present, it is more likely to be constantly present, or, if absent, to be totally absent. At the near measurement, fusion in the primary position was usually present or intermittent, and very rarely absent. In 16% of cases the deviation was greater for distance than for near; in 31%, approximately equal for distance and for near, and in 53% greater for near than for distance. Although some cases fell into the classification of divergence excess and convergence insufficiency, the vast majority did not, and it is urged that the use of such categories be less emphasized.

Constant alternating exotropia constituted approximately 6% of all cases of exotropia; such cases are similar to those of intermittent exotropia. Constant uniocular exotropia constituted about 9% of exotropia. It differs from the other two types in that the strabismus is probably secondary to amblyopia, since a well-defined obstacle to clear vision in one eye can be demonstrated in most cases.

It was also emphasized that exotropia and esotropia differ fundamentally from six points of view: (1) the incidence in the general population; (2) the percentage of incidence of the different types of esotropia and exotropia, i. e., intermittent, constant alternating, and constant uniocular; (3) the etiologic differences of the types; (4) the relation of the strabismus to amblyopia; (5) the role of the refractive error, and (6) the role of heredity.

DISCUSSION

DR. PHILIP KNAPP: It is a moot point whether the literature on convergent deviations is better organized and less confusing than the literature on divergent deviations—certainly it is more voluminous. The fact that different authors use the same terminology to connote different conditions is regrettable, but inevitable, until more facts are known about the binocular neuromuscular mechanism and a uniform terminology is adopted. I use the term intermittent exotropia because it is descriptive of the main characteristics of these cases without the etiologic implication of the term divergence excess. Scobee believed that divergence, being purely passive, is due to relaxation of convergence and so stressed different features. However, the cases and the characteristics are the same. These cases start in the first years of life with an anomalously divergent position of inattention. The course is determined predominantly by how well the fusion mechanism is able to cope with this. I have yet to see a case with relatively equal and useful visual acuity in the two eyes start with only an exophoria for near and lapse into a frank exotropia for distance and near fixation. On the other hand, it has been my experience, both from the history and from observation, that these cases frequently progress from merely an intermittent deviation for distant fixation to a constant exotropia for distance. Then, after a secondary failure of convergence, the near measurement increases until it may equal or exceed that for distance. Naturally, the findings will vary for the age and stage of the process at which one first sees the child. Certainly, in some the deviation remains a phoria for distance and near. In others the condition progresses only part way and then stabilizes. A small minority are unable to cope with the situation at all and it becomes a constant exotropia at an early age. In most cases this process is slower and may never complete the course. One patient who refused surgery had the same measurements and type of deviation after nine years' follow-up. From these observations one may conclude that the condition will not improve, and frequently gets worse.

To rely on the near point of convergence to outweigh the other measurements seems extreme. The near point of convergence will vary tremendously with the examiner and the conditions under which it is measured. It is not uncommon to see notations in charts on successive visits by different observers in which the near point of convergence is remote on one occasion and unlimited on another.

After reading Dr. Schlossman's and Dr. Boruchoff's paper, I took the first 100 cases of divergent deviations, with the same exceptions, from the files of the Muscle Clinic at Presbyterian Hospital. These files go back to 1945, when Dr. Wheeler started the Muscle Clinic. This relatively small number of records was chosen because of the pressure of time. These cases were analyzed for the following factors: age of first visit, age of onset, measurement and type of deviation, near point of convergence, and refractive error. It was impossible to measure the course, as the vast majority either were immediately advised to have surgery, with or without preoperative orthoptics, and were then operated on, or went elsewhere. The average age of the first visit was 6.1 years, including three patients in their 20's. The age of onset was not given in 25 cases. Of the 75 patients noted, 32 had onset soon after birth and only 2 over the age of 4. In 80% the deviation was intermittent. In 27% it was greater for distance; in 62%, equal for distance (20 ft.) and for near (13 in.), and in 11%, greater for near. The near point of convergence

was not recorded in 11 cases. In general, it correlated fairly well, but there were times when it did not. The refractive error based on spherical equivalents revealed 8 cases with 2 D. or more of hypermetropia; 66 cases, between +1.75 D. and —0.75 D., and 25 cases had myopia of 1 D. or more. Moreover, 24 cases had over 1 D. of astigmatism, and 10 had anisometropia of 2 D. or more. The percentage of intermittency corresponds fairly well with the statistics of the authors. The greater percentage of cases with a larger deviation for distance (27 : 16) and the much smaller percentage with a greater deviation for near (11 : 53) may be explained by the younger age of our group and the fact that the near measurement was taken at 33 cm., or 13 in., instead of at 25 cm. These figures lend indirect evidence that the condition does progress with time. Moreover, measurements at 200 ft. or more would further dissociate some cases and increase the number in the group with greater deviation for distance. The refractive findings lend definite evidence that these patients are less far-sighted than average for this age group, according to the figures of Sorsby. The frequency of myopia is not significant, save that in 12 the myopia was over 4 D. Also, the amount of significant astigmatism seems high, as does the incidence of anisometropia. The correction of these errors (myopia and astigmatism) frequently so altered the near measurement that the deviation shifted from the group with equal or greater deviation for near to the group with greater deviation for distance, suggesting that while refractive errors play little part in the etiology of this condition, they greatly affect the near measurement.

In conclusion, I agree that it is important to study each case as to its fusional ability and the depth and extent of suppression, for on these findings one can better base rational therapy than by relying solely on the category into which a case falls on the basis of measurements with the cover test at 20 ft. and 13 in.

Meridional Scleral Resection. Dr. Raymond Meek.

The author presented a new operation, or, rather, a modification of the scleral resection in which he shortens the eye not only from before backward but also from side to side. He prefers a lamellar scleral resection, as outlined by Paufique, rather than a complete resection.

This procedure is of utmost importance in detachments of long standing and in aphakic eyes, in which the retina is apt to be shrunken, and also in high myopia.

In the classic scleral resection, the sclera is resected, two side-to-side incisions being made and the ellipse of sclera between resected. In Meek's procedure, he not only removes a piece of sclera, shortening the eye from before backward, but excises a piece of sclera perpendicular to this first ellipse, as described, so as to shorten the eye from side to side. The sclera outlined is perpendicular to the ellipse first resected, the resection going back as far as possible at any point along the longitudinal area resected so as to be performed behind or in front of the side-to-side resection, or both behind and in front. The piece resected is triangular, with apex behind and the base joining the side-to-side resected area.

A total of 12 cases were presented, with 9 successes and 3 failures. In one of the failures seven holes were observed before operation, and the retina remained in place for one month after the resection, before the detachment recurred. Four of the successful cases were in aphakic eyes, and three were of over six months' duration.

Meek stated that if a patient needs a scleral resection it is imperative that the eye be shortened from side to side, as well as from before backward.

DISCUSSION

Dr. Graham Clark: In the last 10 years we have had a most gratifying increase in the success of our results in the treatment of retinal detachments. The simple detachment has become a matter of surgical routine, and the investigators in the surgery of this disease have been left free to wander into the rather complex field of complicated detachment. By complicated detachment I mean that in which the retina is mechanically prevented from repositioning itself against the wall of the globe. This may be due to two reasons: either traction from the vitreous

body or shrinkage of the retina itself. In these cases diathermy is bound to be a failure. There is no possible way for the retina to reach the choroid in order to obtain the benefit of the adherent diathermy application. Since the retina is completely inelastic and extremely fragile and friable, as all of you who have touched it know, it cannot be pushed or pulled back against the wall of the eye. The vitreous has the unhappy situation of being quite the reverse: It is almost infinitely elastic, and it is also extremely tenacious. You can stretch the vitreous and push the retina back into place, when the retina will allow the stretching, but the vitreous does not give up when you do, and it will pull back again. We have watched such cases in our list of failures. Therefore, with an inelastic and fragile retina held away from the wall of the eye by mechanical forces, there is only one procedure left—the scleral shortening operations.

In this connection, I should like to stress once more what Dr. Meek brought out; that is, all indications for scleral resections are not necessarily alike, and therefore all scleral resections need not be alike. An equatorial resection is, of course, the classic section, with the removal of a section more than half the circumference in the preequatorial area, and this, fortunately, is applicable in a large percentage of cases. The largest group, we have found, has been that in which the traction from the vitreous is the force preventing the retina from repositioning itself against the wall of the eye. That force stems from the base of the vitreous, where the body has shrunken, and extends backward and slightly laterally, but mostly in an anteroposterior direction; and therefore its force can be met by the scleral resection in the equatorial direction. It is probably owing to this sole fact that for so long we were content with the simple equatorial resection.

As you study long-standing detachments, you will notice that there is a very obvious shrinkage of the retina which can be seen clinically, as well as in pathologic sections. The retina is shrunken, and you can sometimes see a membrane stretching between the folds. That is a situation in which a linear attack is useless. That is a situation in which Dr. Meek's procedure has such excellent application. If the retina has shrunken in surface area, there is no linear traction; therefore the remedy must inevitably be directed in both dimensions of the surface, not in the single direction only.

Lamellar resection has been of infinite value in this respect because, while it is possible to exercise the greatest surgical delicacy in cutting around the well-exposed equatorial area, when one proceeds backward into the extreme and strict confines of the orbit, it is impossible to carry out a penetrating resection safely, and a lamellar resection is necessary. I should like to underline Dr. Meek's words to stress the fact that lamellar resection uses the term lamella in its finest sense—the lamella is the smallest possible section one can safely leave behind. I raise that point because of the time-honored dread of a dead space, and I should like to ask Dr. Meek if he has ever seen a rupture of a lamellar resection, as I have.

Dr. Raymond Meek: I have seen a choroidal rupture in a complete through-and-through linear resection, but not in a lamellar one.

Dr. Graham Clark: That is most interesting.

To go back to the bidimensional resection, we started early to use an S-shaped resection. My reason for doing that was to avoid, if possible, the rather difficult situation of an X-shaped or a T-shaped wound, in which two very definite and sharp points must be brought into apposition. The apposition is extremely important because the scleral wound at the end of 14 days is as fresh as it is 14 seconds after you have cut it, and rupture at a weak point is fatal to the eyeball. That S-shaped incision was extremely tedious and difficult, and it was abandoned because its merit did not seem to come up to its difficulty or the danger of the procedure.

It is interesting that a slight variation which fits in with Dr. Meek's procedure is being done by Drs. Swan and Christensen, in Portland, Ore.; it is a chevron-shaped resection. They do it on a lamellar basis. It is not right-angled, but reaches back with the apex toward the limbus, having only one sharp point, which is sutured nicely into a "V." It may be carried back as far as need be and creates a bidimensional effect.

The question of what to do with the lamella brings me into a subject which I should avoid for fear of going on far too long, and that is surface diathermy, and its uncontrollable damage. I should like to suggest, for your consideration, that we borrow from a procedure that has proved excellent, the old Guist procedure. One is so close to the choroid there, and it is so dangerous to turn 200 volts of electricity loose; would it not be better to paint 5% potassium hydroxide on the remaining lamella, neutralize it, and close the wound? One damages nothing but the surface touched, and one gets a good localized choroidal reaction as well.

In the aphakic eye, we have found it helpful to consider two classifications: first, the aphakic detachment, and, second, the detachment in the region of the aphakia. The first is detachment occurring primarily after removal of the lens, and probably due to the mechanical force of the suddenly displaced body of the vitreous. In the second there is a simple horseshoe tear in the preequatorial area, and no particular traction is seen as one examines the vitreous with the ophthalmoscope and slit lamp. The first type demands a scleral resection as a primary procedure; the second, we have found, still responds nicely to well-placed diathermy.

It is only through these surgical refinements and advancements that we shall make progress in this otherwise very discouraging group of detachments. We are a long way from the answer to all of them. We all recognize the early, hopeless massive retraction of the vitreous, the hopeless globes that stand there and virtually stick out their tongues at you! The surgery of these eyes has not yet been answered; but I should like to congratulate Dr. Meek on another approach to this baffling problem, and one which I think is on a sound basis.

Dr. Edward Norton: I have had no personal experience with the variation of scleral resection discussed, but I think that I may be able to answer the question, raised by Dr. Meek, regarding invagination of the choroid. At the New York Hospital-Cornell Medical Center we are using a different technique, based on the work of Dr. Charles L. Schepens, in Boston. Our procedure rests on two essential principles: First, we believe that the resection should be localized directly over the tear; second, we try to invaginate the choroid so as to reduce the necessity for retinal settling.

I should like to demonstrate by means of diagrams why we consider these factors to be important.

In the complicated detachments, the retina is pulled away from the pigment epithelium and choroid. Despite drainage of subretinal fluid, the retina will not settle back on the choroiditis produced by the diathermy, and an almost certain failure will ensue. If a scleral resection is done elsewhere than over the tear, the volume of the globe is decreased and there is probably a greater chance of having the retina come in contact with the thermal choroiditis. However, we believe that one greatly increases the chance of closing the retinal break if the scleral resection is done directly over the tear and if the choroid is deeply invaginated by the use of a polyethylene tube buried in the bed of the resection at the time of closure. By this method, in one procedure, the hole is localized, the volume of the globe is decreased, and the inflamed choroid is brought into close apposition with the detached retina and break. This reduces the necessity for retinal settling, the most unpredictable factor in present techniques.

In answer to Dr. Meek's uncertainty as to what happens to the invaginated choroid, I can say that, with the aforementioned technique, it is visible immediately after operation as a steep elevation. After about a year one sees only the changes in the choroid from the diathermy, as the choroid and retina have usually flattened out. Dr. Schepens has followed some of these cases for two years and has found that the eye tolerates the procedure very well.

Dr. Brittain Ford Payne: It has been a privilege for me to see and hear this new procedure of meridional scleral resection described by Dr. Meek. He asked me to comment on the number of eyes that are consigned to the Eno Laboratory as a result of scleral resection. Only three have reached the laboratory, and none by his technique. It might be added, however, that perhaps sufficient time has not elapsed. Nevertheless, I do think that his operation has a great

deal to offer, much more than the operations which I have performed. My experience has been rather limited, in which about one-third have been moderately successful.

Perhaps the author will give the indications for a scleral resection.

It is obvious that a patient with extreme myopia in which there is a detachment of the retina might need a scleral resection as a primary procedure. Some surgeons prefer a diathermy first. One of my failures was in a one-eyed patient with "bare light perception" on whom a diathermy procedure was performed a year previously. The astonishing thing was that after a year the sclera was necrotic in the region of the diathermy operation. A lamellar scleral resection was performed, but the sutures had to be advanced to hold the edges together.

The most successful result I have had was in an aphakic eye in which a diathermy operation had been unsuccessful. This patient used a +10.00 D. sphere with a cylinder before the resection. After the operation, she accepted a +17.00 D. sphere with a cylinder to obtain 20/30 vision. Scleral resection makes quite a difference in the refraction of the aphakic eye.

When we are doing scleral resections or the conventional diathermy operations for detachment of the retina, few of us pay any attention to the things we learned in our basic course on the microscopic anatomy of the eye. The location of the veins is forgotten, and the long posterior ciliary nerves and arteries are disregarded. Few of us pay any attention to them. When the long posterior ciliary nerves are injured, uveitis and cataract may develop. The eye becomes soft and atrophic and eventually comes to the laboratory.

Dr. Meek mentioned his resection operation on television and its success. For the benefit of those who participated in the television program of the International Congress, Dr. Benedict, the secretary-general, has written a letter expressing his appreciation to all the participants.

A total of 37 major operations were performed at the New York Eye and Ear Infirmary, and, despite our inexperience, there were no accidents, no hemorrhages, and no infections, and, so far, none of the eyes are in the laboratory.

DR. RAYMOND MEEK: May I ask Dr. Clark to discuss the polyethylene tube? He has had much more experience with this than I have; he has worked with Dr. Schepens and knows more about it.

DR. GRAHAM CLARK: I would like to correct one impression: I have not had the privilege of working with Dr. Schepens, but I have observed his work. I find it extremely interesting to compare, or perhaps I should say contrast, the two methods, and I think it is an extremely healthy and very stimulating atmosphere in which we are working. We have in these two procedures two distinct approaches to what appears to be the same problem. The scleral buckling, as it is called, has as its purpose a twofold mission: first, to bring the wall of the eye to the retina, and, second, to close the hole. The other resections which Dr. Meek and I have referred to have another mission—to create a simple detachment out of a complicated one. This is the sole purpose of a simple scleral resection—to make it possible for the retina to reach the wall of the eye. It is then handled as a simple detachment; the hole is closed with diathermy, and that is the end of the procedure.

The buckling is contrary to a great deal of surgical teaching. First, one is not supposed to create a dead space. Another surgical principle which is seemingly violated is the implantation of a foreign body. A foreign body implanted in a dead space immediately causes one to raise one's mental eyebrows, but the evidence of time has shown they can come back down again—apparently nothing happens. It is most interesting, and I await eagerly some animal experimentation. Is our old rule as fallacious as it seems to be? It is interesting to watch the two procedures, and, as usual, we shall probably end up halfway between, or with a third.

DR. RAYMOND MEEK: I want to thank Dr. Clark, Dr. Norton, and Dr. Payne for their charitable discussions.

In answer to Dr. Payne's question about whether in a case of high myopia I should do a meridional lamellar scleral resection first, I believe that in such a case I should do it first, for one reason that he discussed; that is, if one does diathermy

first and then has to go back and do a meridional resection, one has a sclera that has been damaged and it is very difficult to make the sutures hold. The conjunctiva is also bound down with adhesions and most difficult to free, and the chance of success is in my opinion much better.

I feel very apologetic in giving my results this evening. I should have felt better if I had had more failures, because then I should feel that they would be believed more readily; but I have honestly reported these cases with the results as I have found them.

John M. McLean, M.D., *Chairman*

Joseph Laval, M.D., *Secretary*

Dec. 20, 1954

Effect of Version and Vergence Movements on Ocular Tension. DR. JOSEPH I. PASCAL.

When the eyes make a version movement from the primary position to an oblique secondary position they undergo some (false) torsion. The kind and amount of the torsion can be calculated. When the eyes converge they also undergo some torsion, except when they converge in a plane some 30 to 45 degrees below the horizontal, when the convergence is not associated with any torsion. This is what Helmholtz called the primary position for convergence. (A fuller discussion of these two types of torsion was presented.)

A curious circumstance arises when one compares the torsion undergone by one eye (*a*) when it is associated with the other eye in a version movement, and (*b*) when it is associated with the other eye in a convergence movement. It is found that the eye while maintaining the same visual direction is intorted in one case and extorted in the other case. The mechanism of this reversal of torsion is explained by reference to the secondary actions of the several muscles involved in the movement. (An illustrative case which vividly brought out this effect was included.)

DISCUSSION

DR. ADOLPH POSNER: For several years I have been interested in the effects of torsion on the axis of astigmatism. Although this is an old subject, very few ophthalmologists pay much attention to the effects of torsion in the various positions of gaze on the refractive correction. What I am particularly interested in is the effect of head tilt movements on the axis of astigmatism. It is well known that when the head is tilted to one side the eyes undergo a torsion movement in the opposite direction. Since the spectacles are fixed in position with reference to the head, the axis of the cylindrical lens which is adjusted correctly for the primary position is no longer coincident with the axis of astigmatism.

The study of torsion movements which Dr. Pascal has reported is extremely interesting. I am referring particularly to the extorsion of the eyes in convergence movements when the gaze is directed upward. It is important to bear this point in mind because, while it may seem esotoric to the average ophthalmologist, it can play a role in the comfort of the patient who wears a high astigmatic correction. I am glad that Dr. Pascal brought this question up, even though I do not think that either he or anyone else can solve the problem in a practical manner. I hope, however, that Dr. Pascal's report may stimulate further investigation along this line. For example, it would be of interest to study some of the refractive problems in patients who are dissatisfied with their glasses. Each one of us knows many patients with high astigmatic correction who prefer to go without their glasses even though their vision is greatly improved with their glasses. It is possible that by taking the torsion movement into consideration we could adjust the axis of their lenses in such a way that they would gain more comfort from wearing them.

DR. JOSEPH I. PASCAL: Dr. Posner has brought up a very good point bearing on this subject, namely, the effect of the torsion movement on the axis of astigmatism, especially high degrees. As he said, as far as the practical solution goes, there is as yet no fully satisfactory answer, since the amount and kind of torsion constantly vary with the direction of gaze, the inclination of the head, and so on.

But this is a point to be taken into consideration, especially in patients who have to do near work above the level of the eyes. Marked convergence in this upward gaze will require a change in the astigmatic axis from that found monocularly with the eyes in the primary position.

Doing close work above eye level has always been rather fatiguing. The etiology has been explained in many ways, but I believe the main element in this discomfort is the marked binocular extorsion found in this position. That is why, as Dr. Posner has mentioned, some people may be better off not using their cylinders in these special tasks, but using only spheres, the equivalent sphere, though vision may be slightly reduced. I hope Dr. Posner's plea for a research study of some of the problems herein raised may bear fruit.

Leber's Disease with Recovery. Dr. Elizabeth F. Constantine (by invitation).

The literature was reviewed in some detail. A case of Leber's disease in a 17-year-old white youth was presented. When he was first seen, two and a half months after the onset of blurred vision, R. E. V. was 20/200 eccentrically and L. E. V. was 20/200 centrally. Both discs showed temporal pallor, and there were marked central scotomata. X-rays of the sella turcica and the optic foramina were negative. Vision fell in a week to 1/200 in the right eye and within seven weeks to 5/200 in the left eye, and remained essentially unchanged until one year from his first visit, when it was found to have improved to 20/100 and 20/200 in the right and left eyes, respectively. Four months later it was 20/20+3 and 20/15—2, and fields showed marked improvement with breaking up of the scotomata. Temporal pallor of both discs remained unchanged.

From relatives a pedigree was obtained, covering five generations and 100 members. Of these, 17 were reported to be affected by Leber's disease, and all were males. One maternal uncle was examined and found to have reduced central vision and marked optic atrophy in both eyes. Four of the affected members of the family were said to have recovered partially or completely.

The only treatment this patient received consisted of injections of vitamin B_{12} over a period of six weeks after he was first seen, and it was discontinued from seven to nine months before recovery commenced. It was not thought to have influenced the course of the disease in any way.

The case was presented because it offered fairly definite and recent proof of recovery.

DISCUSSION

Dr. Frank D. Carroll: Dr. Constantine has, I believe, made an important contribution and has well outlined the literature on this subject. At the chairman's request, I have reviewed the records of my patients with Leber's disease and find 21 such persons in 15 unrelated families. Dr. Constantine mentioned that one author stated that in the same family the age of onset tends to be the same. That may be true frequently, but in one of my families which could be well traced over a period of three generations, the age of onset varied from infancy to age 57. The disease occurs at any age and must be considered in all patients with optic neuritis or optic atrophy in whom another etiology is not discovered. In taking the family history it is well to inquire specifically about the mother's brothers; almost any type of inheritance is possible, but, as you know, the disease is frequently inherited by males through unaffected females. Seventeen of my patients were males, 4 females.

The vision of affected members of the same family may vary widely. Most may have their vision so impaired that it is less than 10/200, but others will have vision as good as 20/40. Eight or 10 of my patients are able to operate newsstands in New York City. None are totally blind.

Dr. Constantine stresses that her patient, over a period of many months, made a spontaneous recovery without therapy. Just consider what an erroneous impression might have been placed in the literature if the patient had recovered while receiving cortisone, vitamins, or vasodilators, and if the patient were not under the observation of as understanding a physician as the speaker. Some of my

patients with optic neuritis of unknown etiology have recovered spontaneously one and, infrequently, even two years after onset of the disease. Unfortunately, none of my patients with Leber's disease have improved, and if Dr. Constantine can continue this record I shall be glad to send her my cases in the future.

Effect of Succinylcholine on Intraocular Pressure. DR. HARVEY A. LINCOFF and DR. CHARLES H. ELLIS.

Succinylcholine is a new curare-like agent that has the unexpected effect of raising intraocular pressure. In experiments on animals, this pressure rise was shown to vary quantitatively with the dose. Maximum rises in animals were of the order of 12 to 18 mm. Hg. The pressure rises seen in human subjects were less regular, but approximated those seen in animals. Deep anesthesia was found to inhibit the intraocular pressure rise in humans.

The mechanism of the pressure rise was explored. Changes in arterial pressure and anoxia were ruled out. A slight rise in venous pressure was seen to occur with succinylcholine, and it was felt that this might play some part in the rise in intraocular pressure. In further experiments it was found that while the other skeletal muscles were relaxed following the injection of succinylcholine, the ocular muscles were in a state of sustained contracture. The rise and fall in pressure was shown to be coincident with the contraction and relaxation of the extraocular muscles.

It was concluded that succinylcholine raises intraocular pressure, and that the mechanism is probably contracture of the extraocular muscles.

DISCUSSION

DR. A. GERARD DEVOE: This may seem like a somewhat theoretical subject, but the material presented is of considerable importance to us clinically. I would like to discuss this very briefly from two standpoints—one, the use of curare-like agents in cataract extraction, and, two, the specific use of succinylcholine. The use of curare-like agents was popularized in this country by Dr. Kirby. Dr. Kirby was a superb technician, as you know; to the best of my knowledge no fatalities occurred during his use of the drug. That has not been the case with other operators, however. Several serious problems arise with the use of curare-like agents. There is always the possibility of respiratory paralysis, which is no problem in a general surgical procedure, where the patient is intubated and a first-class anesthetist is on hand to handle the emergency. The average cataract patient is not intubated when we start to operate, nor would this be desirable in the middle of a cataract extraction. Secondly, complete circulatory collapse, although uncommon, may occur. If it does, we are practically helpless; we have no suitable antidotes for succinylcholine. We have antidotes for curare, such as neostigmine, but for the depolarizing drugs like succinylcholine these make the situation worse. Therefore we have to think seriously before we embark on the use of curare-like agents in cataract surgery. We have to weigh the possibility, which is probably not great in the average, properly prepared cataract patient, of losing vitreous against the possibility, which I think, again, is not great, of death; and you will have to be the one to decide whether the risk is worth it.

With succinylcholine, Dr. Lincoff has demonstrated that there is a well-marked contraction of extraocular muscle. He did not tell you, but he has done cataract sections in cats, given the drug, and produced prolapse of the iris. He has also produced loss of vitreous after giving the drug. With this drug specifically we should be extremely careful. I know that it is used by the anesthetist, sometimes without our knowledge, in cases where the curare-like agents are deemed advisable. Personally, I would not choose to use any of these agents for cataract surgery.

Dr. Lincoff says he did not think it unsafe to use succinylcholine in glaucoma. I think that is probably true in deep-chamber glaucoma, but in a patient with a narrow angle, sudden expression of fluid from the anterior chamber by extraocular muscle contraction might very easily precipitate an acute angle blockage. Such has not yet appeared clinically, to the best of my knowledge.

I again want to thank Dr. Lincoff for contributing a great deal to this subject, which is important both clinically and as a tool for further investigation.

DR. DAVID J. IMPASTATO: Dr. Lincoff very courteously invited me to say a few words. As a psychiatrist there is nothing that I might add or discuss from the ophthalmologic point of view. Psychiatrically speaking, however, we are intensely interested in succinylcholine, as it is helping us to eliminate the commonest complication of electroshock therapy, namely, fractures.

We are from time to time called upon to administer EST to patients with eye conditions and very often call on the ophthalmologist to tell us if we may proceed. There is a need for close cooperation between the psychiatrist and the ophthalmologist in the treatment of such patients.

Unfortunately, there is little in the psychiatric literature regarding electroshock therapy in patients with eye conditions. There are a few studies regarding the intraocular pressure which has been found to be raised following electroshock therapy, and some ophthalmoscopic observations in which it has been shown that the retinal vessels at first are narrowed and later are dilated during the convulsion. Observations have also been made on the size and behavior of the pupils during electroshock therapy. The literature is practically silent as to eye complications during electroshock therapy except for subconjunctival hemorrhages, which occur perhaps in 1 out of 200 patients. These are benign and usually disappear even though treatment is continued. Hemorrhages have been reported in other parts of the body and other organs, such as the stomach, thyroid gland, etc., and it is possible that they have occurred in the retina. However, we have no record of this, and I cannot recall having had such a complication in over 5,000 patients, so that if retinal hemorrhages do occur, they are extremely rare. Apparently patients with retinal hemorrhages secondary to hypertension have been safely treated with electroshock, and in one reported instance the hemorrhages receded as the patient's mental condition improved and his blood pressure came down.

Dr. Lincoff stated that glaucoma is not a contraindication to electroshock therapy. We have recently treated a patient with recurrent episodes of detached retina. At the time of treatment the retina was attached. No complications occurred.

I wish to congratulate the authors on their carefully prepared and presented paper.

DR. LUDWIG VON SALLMANN: This exciting paper is of particular interest to me because I have been approached three times in the last six months by the anesthetist of the Institute of Ophthalmology with the question whether succinylcholine interferes with the intraocular pressure when it is used in general anesthesia. I have no experience with this muscle relaxant, but I thought of a possible peripheral vasodilating effect similar to that of acetylcholine and I planned to do some experimental work with this compound. Now the reported pertinent study provides important information. The results presented by Dr. Lincoff and Dr. Ellis seem to exclude such a neurovascular mechanism for the observed dramatic rise of intraocular pressure, although dissociation of eye pressure and blood pressure changes is known to occur after intravenous injection of acetylcholine. The assumption that a contracture of extraocular muscles produced the transient elevation of the eye pressure appears well documented, particularly by the magnitude of the responses (16 mm. in cats). The puzzling combination of a general blockade of neuromuscular transmission synchronous with contracture of some orbital striate muscles leads to the question whether the latter effect is brought about by a central drug action or can be explained by differences in the neuromuscular junction of the respective muscles. I would like to ask Dr. Lincoff, therefore, about the advisability of determining the action potentials at one of the most accessible nerves of the striate muscles in the orbit during the general neuromuscular block and the rise of the intraocular pressure. Principally, I would agree with Dr. DeVoe in considering as unwarranted in patients with narrow-angle glaucoma or the predisposition to this disease the use of a drug from which an even short-lasting eye pressure increase of 20 to 40 mm. Hg may arise.

DR. HARVEY A. LINCOFF: In regard to the question of whether there is a central mechanism or not, I cannot answer except to say that the rotations of the globe are typical of a peripheral mechanism. Perhaps Dr. Ellis will have a suggestion.

In regard to whether a vascular phenomenon, namely, arterial pressure, is significant, I did not point out the tracing of our experiment on arterial pressure, which was actually part of the first slide. However, it showed that we could not produce any rise in arterial pressure with ordinary doses of succinylcholine, although we consistently got a rise in intraocular pressure with these doses. There is a report that doses of the order of 100 times the respiratory paralysis dose of succinylcholine will cause a rise in arterial pressure. The question of whether succinylcholine will produce a rise in blood pressure is, I know, in dispute, and several people in the city are working on this matter. We did not expect arterial pressure, if it rose at all, to cause a rise in intraocular presure, because we thought its effect would be dampened by the end-arterioles in the eye.

In regard to our evidence that contraction of the extraocular muscles is the major precipitating factor in the rise in intraocular pressure, I realize that our proof is largely indirect, except for the instance where we withdrew a muscle from the orbit and observed it to draw back into the orbit upon the injection of succinylcholine. Our conclusion that the muscles contract rests largely on the fact that the eye rotates in the direction of the unsevered muscles in the experiments previously referred to. Further, I would like to mention that when we seized the globe with a forceps after the injection of succinylcholine, we could feel it actively rotate to a position of immobility. Direct proof that the muscles contract would consist of a nerve muscle preparation which could be observed, and electromyography. These experiments are contemplated.

DR. CHARLES H. ELLIS: I think the only thing I can say about the central effect is that we do not know. There seems to be a little evidence that succinylcholine, like the other muscle relaxants, shows a possibility of a central action, at least so far as respiration goes. We do not know whether it is central or not; we have not investigated it that far, but we do know that there can be respiratory arrest with a perfectly functional phrenic nerve. In other words, stimulating the phrenic nerve will result in contraction of the diaphragm; so there is something which is lacking above the myoneural junction. That shows up only with excessive doses of succinylcholine, and we are not too sure whether it is actually a central depression or not. How that applies to intraocular pressure we have no information; so we cannot make any answer at all.

In regard to the question of the peculiar type of muscle apparently present in the extraocular eye muscles, we do know that the frog's rectus abdominis muscle goes into slow contracture, with a slow onset and rather long-lasting shortening, in the presence of acetylcholine, of succinylcholine, and of decamethonium (Syncurine). In other words, substances acting like acetylcholine cause the frog's rectus abdominis to go into this contracture. Whether or not the eye muscles are similar to that in their response to succinylcholine we have not determined; in other words, we have not set up a muscle-nerve preparation where we can stimulate a nerve and record the contraction of one of the eye muscles. That is an experiment which should be done, but we have not yet done it. We do know that when we take hold of the globe with forceps it can be moved around quite easily. After succinylcholine it becomes almost locked and only very restricted movements are possible; so it looks very much as though there develops either a sustained contraction, which is doubtful, or a contracture, which seems much more likely in the case of the extraocular muscles. This report is preliminary. We have not done all of the experiments we should be doing, but we do know that there is a marked increase in intraocular pressure, and we are sorry to see it with succinylcholine, because we would like to see succinylcholine an all-purpose muscle relaxant. Unfortunately, there is this response which has occurred every time in the cats and dogs we have worked on, and, as Dr. Lincoff pointed out, it has occurred in by far the majority of the 75 electroshock patients. Since it is apparently a rather commonly occurring phenomenon, we felt, just as a matter of putting it on record, that it should be reported that succinylcholine does cause a rise in intraocular pressure. As to why we are still more or less in the dark, but it apparently does occur.

Test for Active Eye Changes of Graves's Disease Using Triiodothyronine and the Twenty-Four-Hour Uptake Method. DR. SIDNEY C. WERNER.

Patients without thyroid disease show a sharp decrease in I^{131} uptake when given triiodothyronine or thyroid; little change in uptake is induced in patients with active Graves's disease. This reaction is so consistent as to provide a test (*Bull. New York Acad. Med.*, to be published). By means of this test, patients with active eye signs of Graves's disease can be distinguished from patients with inactive eye signs remaining after the successful treatment of Graves's disease, and from patients with eye changes simulating Graves's disease, such as pseudotumor or lymphoma of the orbit. These last two groups show a normal response, with decreased uptake to triiodothyronine administration. Finally, a group of euthyroid patients with the early eye changes of Graves's disease have been identified and found to give the same limited response to triiodothyronine as patients with outspoken hyperthyroidism (*Am. J. Med.*, to be published). The clinical characteristics of these patients has been presented.

COLLEGE OF PHYSICIANS OF PHILADELPHIA, SECTION ON OPHTHALMOLOGY

Edmund B. Spaeth, M.D., *Chairman*

William E. Krewson 3rd, M.D., *Clerk*

Oct. 21, 1954

Hyaluronic Acid and Hyaluronidase in the Aqueous Humor and the Anterior Chamber Angle. DR. ERNST BARANY, Upsala, Sweden.

The resistance to flow through the chamber angle of excised animal eyes is reduced to about half by small amounts of testicular or bacterial hyaluronidase. This indicates the presence of mucopolysaccharides in the outflow barrier. A search was made for hyaluronidase in the aqueous humor, which might have been an important controlling factor for the outflow resistance. No evidence for the existence of the enzyme in the aqueous was found. The mucolytic properties of the aqueous in vitro are explained by its ascorbic acid content.

Lowering the intraocular pressure by unilateral carotid ligation in rabbits leads to an increase in the resistance of the angle. Increasing the intraocular pressure by venous obstruction decreases the resistance of the angle. The resistance of normal rabbit eyes is unaffected by prolonged treatment with progesterone ointment but lowered by cortisone and cortisol.

Edmund B. Spaeth, M.D., *Chairman*

William E. Krewson, 3rd, M.D., *Clerk*

Nov. 18, 1954

Evaluation of Certain Glaucoma Operations, with Especial Reference to Cycloelectrolysis, Cyclodiathermy, and Iridocorneosclerectomy: The Seventeenth Annual de Schweinitz Lecture. DR. CONRAD BERENS, New York.

The choice of an operative procedure for the control of tension in glaucoma should be carefully considered, not only for its mode of action but also for the over-all long-range performance of the operation. The evaluation of the procedure should include the control of tension within normal limits without or with the use of miotics, the increase or preservation of the preoperative visual fields and visual acuity postoperatively, and the immediate and long-range postoperative complications.

The compilation of 239 eyes affected with all types of glaucoma, in which cycloelectrolysis was performed and postoperative observations were made from two to nine years, revealed that 32% of these eyes were controlled without miotics and 33% with miotics, for a total of 65% of the eyes in which tension was controlled under 25 mm. Hg (Schiøtz). Vision improved or remained unchanged in

62% of 225 retained eyes in this series. One per cent of the eyes became atrophic. Other complications encountered were iridocyclitis, in 1+%, and absolute glaucoma, in 3%, of the eyes.

Anterior cyclodiathermy was performed in 264 eyes affected with all types of glaucoma and observed postoperatively for from 1 to 14 years. Four per cent of the eyes were controlled without miotics and 24% with miotics, for a total of 29% of the eyes in which tension was controlled under 25 mm. Hg (Schiøtz). Vision in 224 retained eyes improved or remained unchanged in 17% of this series. The more serious postoperative complications observed in this series of 223 retained eyes include atrophic globes, 11%; phthisis bulbi, 4%; hemorrhage of the anterior chamber, 9%; vitreous hemorrhage, 3%; uveitis, 4%, and cataract, 2%.

The prognosis following retrociliary diathermy in 127 eyes observed postoperatively for from one to four years is poor. Only 4% of the eyes were controlled without miotics, and 22% with miotics, for a total of 26% of the eyes in which tension was controlled under 25 mm. Hg (Schiøtz). Vision improved or remained unchanged in 35% of 114 retained eyes. The postoperative complications include atrophic globes, 6.5%; phthisis bulbi, 2%; hemorrhage in the anterior chamber, 5+%; vitreous hemorrhage, 1%; iridocyclitis, 1%; absolute glaucoma, 1%, and panophthalmitis, 2%.

Of the two most used procedures designed to diminish the formation of aqueous, cycloelectrolysis seems to be more effective than cyclodiathermy, with fewer postoperative complications. The long-range prognosis following anterior cyclodiathermy is discouraging, and the results of retrociliary diathermy are poor, with a high percentage of atrophic eyes resulting from both of these procedures. Because most increased tension results from diminished outflow, and the hypotensive effect of operations like cyclodiathermy and cycloelectrolysis is often of short duration, these procedures should be reserved for cases in which filtering operations are contraindicated, or when outflow following a filtering procedure is mildly inadequate.

Evaluation of operations producing subconjunctival filtration showed that the results following iridocorneosclerectomy were superior to those following simple iridencleisis and iridencleisis combined with sclerectomy.

In a series of 511 eyes observed for from 1 to 24 years following iridocorneosclerectomy for all types of glaucoma, tension was controlled under 25 mm. Hg without miotics in 30% of the eyes, and with miotics in 47%, for a total of 77% of the eyes controlled. Vision improved or remained unchanged in 56% of the 487 retained eyes. Atrophy of the globe occurred in one eye; phthisis bulbi, two eyes; absolute glaucoma, three eyes; iritis, two eyes; iridocyclitis, one eye; uveitis, two eyes; vitreous prolapse, one eye; synechiae, one eye; cataract, one eye, and hemorrhage in the anterior chamber, 1% of the eyes.

The results of iridencleisis for various types of glaucoma in 501 eyes, observed for from 1 to 14 years postoperatively, revealed that the tension was controlled without miotics in 14%, and in 38% with miotics, making a total of 52% in which tension was controlled under 25 mm. Hg (Schiøtz). Vision was improved or remained unchanged in 40% of 481 retained eyes. The complications include atrophic globes, 1%; phthisis bulbi, 2%; absolute glaucoma, 3%; iridocyclitis, 1%; cataract, 4%; dislocated lens, 1.5%, and hemorrhage into the anterior chamber, 8%. Severe uveitis which was diagnosed as possible sympathetic ophthalmitis was reported in two eyes, a case of questionable sympathetic ophthalmitis in a third patient, and proved sympathetic ophthalmitis in a fourth.

The postoperative results of iridencleisis combined with sclerectomy in 100 eyes affected with chronic simple glaucoma, observed for from one to nine years, show that tension was controlled without miotics in 29% of the eyes and with miotics in 44%, making a total of 73% of the eyes in which tension was controlled under 25 mm. Hg (Schiøtz). Vision in 96 retained eyes showed improvement in 28%, and was unchanged in 27% of the eyes.

When a fistulizing operation is indicated and especially when cycloelectrolysis is contraindicated, iridocorneosclerectomy seems to be the operation of choice and has controlled tension and preserved visual acuity over a long period of time, with a minimum of complications encountered in eyes observed for from 1 to 24 years.

Correspondence

NEW PREPLACED CORNEAL SUTURE

To the Editor:—With reference to the "New Replaced Corneal Suture" described by A. Lachner, M.D., in the July, 1954, issue of the ARCHIVES, page 129, and discussed by M. A. Shah, M.B., M.S., in the December, 1954, issue, page 970, and by F. A. Williamson-Noble in the January, 1955, issue, page 192, I should like to say that during my residency, in the fall of 1953, my interest in the preplaced suture, after reading McLean's article in the ARCHIVES (**23**:554 [March] 1940) lead me to arrive at the same technique as that described by Dr. Lachner, but for the one variation which I feel completes the technique by facilitating grasping of the suture and eliminating its being frayed. This variation consists in not being anxious to run the entire needle completely through to the scleral side of the limbus but in stopping the hub of the needle right in the groove, depressing the exposed part of the needle toward the globe, and backing the hub up through and out of the groove until about one-half of the proximal aspect of the needle is in view. Then, by employment either of a blunt iris hook or of a Wiener corneal hook, the needle alone is hooked close to the limbal groove, and then this needle is brought through to the scleral side of the limbus. The suture will always be found on the hook. It is not a good practice to try fishing for the taut suture at the posterior aspect of the needle, for this maneuver also tends to fray the suture material, or even pull it off the eyeless type needle. I have found that the #81 Grieshaber corneal needle is preferable to the eyeless type because the thread may fray on the latter as the hub is emerging from the groove. For several reasons, I prefer to use the Verhoeff tract technique and have found that if the surgeon will dull the tip of one of the 6-0 needles in each of the double-armed sutures and then resterilize the units, he will find the rethreading much easier because the needle tip does not get caught along the canal walls. A #81 Grieshaber needle can then be used to replace the dull needle in order to pass it through the conjunctival flap.

Perhaps of some interest to other ophthalmologists because of usefulness, low cost, and the ease of operation and installation is the employment in the office or clinic of a foot switch #491-S Linemaster Compact (cost approximately $4), manufactured by the Linemaster Switch Corporation, Woodstock, Conn. This foot switch I have placed into the line, within easy reach of my foot, to both my slit-lamp biomicroscope and lensometer. These two instruments are on only when the foot switch is continuously depressed and are automatically turned off when I remove my foot as I walk away from the instrument. The foot switch is approximately $2\frac{1}{2} \times 2\frac{1}{2} \times 1$ in. Once one has used this switch, one wonders why the instrument doesn't come equipped with it.

Submitted for what it might be worth to ophthalmology is the following: There are to be seen in the literature discussions on sterilization methods for the tonometer and on instances in which one hesitates to utilize this instrument. I feel that a satisfactory way of taking readings is to cut a small portion of a condom into the following shape to fit the palpebral fissure and place this onto the patient's globe with the $\frac{1}{2}$ in. tag of the rubber hanging over the lateral commissure. I have not been able to detect any appreciable difference in readings with and without the latex product on routine examinations; however, a careful study in a large clinic would be desirable. The rubber pads can be kept sterile in benzalkonium (Zephiran) chloride solution 1:1000. If this idea has any merit, the latex companies may be influenced to stamp out these rubber pads with more manageable

tags. Utilizing but one tonometer, a practitioner could screen a fair number of patients a day, maybe paving the way to a Glaucoma Detection Week, just as we now have Diabetes Detection Week. Has the shoe found a useful purpose on a different foot?

LLOYD M. HORLICK, M.D.
82 Court St.
Portsmouth, N. H.

UNILATERAL BLINDNESS DURING ANESTHESIA

To the Editor:—I have just finished reading the article in the December, 1954, issue of the ARCHIVES by Drs. Robert W. Hollenhorst, Henrik J. Svien, and Clair F. Benoit, entitled "Unilateral Blindness Occurring During Anesthesia for Neurosurgical Operations," and it recalled to mind an incident which occurred more than 30 years ago, when I was a resident at the Manhattan Eye, Ear and Throat Hospital. A lacrimal gland was excised on the service of Dr. Herbert Wootton, and, because of troublesome bleeding, a pressure bandage was applied. When the bandage was removed at the end of 48 hours, the patient remarked that she could not see. Examination of the eyegrounds revealed no apparent abnormality, but as the days went by the patient did not recover her vision, and optic atrophy gradually evolved.

In February, 1924, when I was doing some postgraduate work in Vienna, I asked Docent Guist if he had ever seen such a case, and he immediately became excited, saying that within that past year they had had three such incidents in the Allgemeines Krankenhaus. It was his theory that bleeding in the orbit, pushing the globe forward against a pressure bandage, elevated the intraocular pressure to such an extent that the retinal circulation was temporarily suspended, and that the nuclei in the nerve center of the retina died as the result of the long-continued ischemia.

I have always borne this in mind and, avoiding the use of pressure bandages on healthy eyes, have not since seen a similar case.

W. GUERNSEY FREY, M.D.
121 E. 60th St.
New York 22

INTRAOCULAR PENETRATION OF LOCAL HYDROCORTISONE AND CORTISONE

To the Editor:—Weimar and Leopold, in a very constructive article in the November, 1954, issue of the ARCHIVES, point out that hydrocortisone acetate injected subconjunctivally did not penetrate the eye in high levels, with the test employed.

In my article in the *American Journal of Ophthalmology* in April, 1954, it was pointed out that "hydrocortisone acetate was inferior to the free-alcohol form of hydrocortisone in the treatment of eye disease by subconjunctival injection. A combination of the hydrocortisone acetate and free-alcohol form of hydrocortisone has proven very satisfactory."

Experience with systemic hydrocortisone indicates a superiority over cortisone in intraocular inflammations. Peculiarly enough, hydrocortisone acetate has a marked superiority over the virtually inactive cortisone acetate when employed as an ointment in the treatment of dermatological conditions of the skin or mucous membranes.

The inferiority of hydrocortisone acetate to cortisone acetate by subconjunctival injection may be due to one of many factors: (1) Too rapid systemic absorption of the hydrocortisone acetate may occur; (2) hydrocortisone acetate may be changed to something else within the eye, or (3) the hydrocortisone acetate may be absorbed into the eye in very small amounts over a longer period of time.

The free-alcohol form of hydrocortisone was effective clinically, but over too short a period. Therefore, it was combined with what was thought to be the longer, slower-acting acetate.

One cannot apply the experimental or clinical evidence gained with hydrocortisone acetate to any other form of hydrocortisone. To date I have employed three other forms of hydrocortisone, no two of these functioning alike clinically.

Too frequently ophthalmologists overlook the fact that the dose and route of administration determine the efficacy of the steroid used.

The role of steroid therapy in ophthalmology is an important one. However, there is very little question that hydrocortisone and cortisone acetate will be superseded by still other forms, and these, similarly, in their turn.

DAN M. GORDON, M.D.
133 E. 58th St.
New York 22

To the Editor:—I appreciate the remarks made by Dr. Gordon, stimulated by our paper. I believe his discussion may prove helpful in the handling of steroids in ophthalmology.

IRVING H. LEOPOLD, M.D.
1930 Chestnut St.
Philadelphia 3

A PHYSIOLOGICAL UNIT OF CONVERGENCE

To the Editor:—Convergence is a binocular function, as there can be no convergence in a one-eyed person. The angle formed by the two visual axes at the point of fixation is the (static) angle of convergence. It would therefore seem logical to define the unit of convergence, the meter angle, in a binocular sense. This has now been brought about by the recent decision of the Committee on Optics of the American Academy of Ophthalmology. They define the meter angle as the angle formed by the two visual axes at a point of fixation 1 meter from the center of the interocular base line.

The original definition of Nagel used one visual axis as the measuring line; that is, the point of fixation was 1 meter away as measured on the visual axis. Use of the midline, that is, the line from the midpoint of the interocular base line to the point of fixation, is preferable, as it can be used much more widely. If the point of fixation is on the median plane, then the midline becomes the median line. When the point of fixation is not in the median plane, say, it is to the right or the left of the median plane, then the midline is not the median line. But it can still be used conveniently as the measuring line.

One objection to the meter angle as a unit of measurement that has been raised at one time or another is that in actual degrees the angle corresponding to, say, 5 meter angles is not equal to five times 1 meter angle. It is slightly less. But the difference is extremely small, a matter of 6 minutes of arc in an angle of convergence of over 17 degrees. By direct computation, the angle corresponding to 5 meter angles (for a P. D. of 60 mm., point of fixation in the median plane) is 17°14′. The angle as found by multiplying the unit (i. e., $5 \times 3°8'$) is 17°20′.

When the point of fixation is to the right or the left of the median plane, the meter angle, in degrees, is slightly less than when the point of fixation is on the median plane. But here, too, the difference is very small. For example, the degree value of 5 meter angles for a point of fixation 15 degrees to the side of the median plane is 16°30′ as against 17°14′ on the median plane. Thus, use of the midline as above defined for measuring convergence in meter angles, wherever the point of fixation may be, is really a simple and practical method. This concept of the meter angle is bound to supersede the idea of a monocular meter angle as often defined, using the median line as one of the sides of the angle.

JOSEPH I. PASCAL, M.D.
41 W. 96th St.
New York 25.

News and Comment

EDITED BY DR. W. L. BENEDICT

SOCIETY NEWS

Pennsylvania Academy of Ophthalmology and Otolaryngology.—The 13th annual meeting of the Pennsylvania Academy of Ophthalmology and Otolaryngology will be held May 18 to 21, 1955, at the Hotel Traymore, Atlantic City, N. J. This meeting is being held jointly with the New Jersey Society of Ophthalmology and Otolaryngology and the West Virginia Academy of Ophthalmology and Otolaryngology.

Dr. James H. Delaney, president of the Pennsylvania Academy, will preside. The guest of honor for the meeting will be the President-elect of the American Medical Association, Dr. Elmer Hess.

The meeting will consist of study clubs and general sessions. The faculty for the study clubs will be members of the societies. Papers at the general sessions will be given by members of the societies and invited guests.

Those invited to participate are as follows:

Ophthalmology:

Samuel Blank	Chief, Department of Anesthsia, Wills Eye Hospital.
F. Bruce Fralick	Professor of Ophthalmology, University of Michigan.
Glen G. Gibson	Professor of Ophthalmology, Temple University School of Medicine.
Peter C. Kronfeld	Professor of Ophthalmology, University of Illinois College of Medicine.
Edmund B. Spaeth	Attending Surgeon, Wills Eye Hospital. Professor of Ophthalmology, Graduate School of Medicine, University of Pennsylvania.
John M. McLean	Professor of Ophthalmology, Cornell University Medical College.
James S. Shipman	Attending Surgeon, Wills Eye Hospital. Clinical Professor of Ophthalmology, Graduate School, University of Pennsylvania. Associate Professor of Ophthalmology, Jefferson Medical College.
I. S. Tassman	Attending Surgeon, Wills Eye Hospital. Associate Professor of Ophthalmology, Graduate School, University of Pennsylvania.
P. Robb McDonald	Attending Surgeon, Wills Eye Hospital. Ophthalmologist to the Lankenau Hospital.
John S. McGavic	Chief of Ophthalmology, Bryn Mawr Hospital. Associated Professor of Ophthalmology, Hahnemann Medical College.
Harvey E. Thorpe	Chief, Department of Ophthalmology, Montefiore Hospital.
Edwin B. Dunphy	Professor of Ophthalmology, Harvard Medical School. Director, Massachusetts Eye, Ear, Nose and Throat Hospital.
Charles I. Thomas	Assistant Professor of Ophthalmology, Western Reserve, University School of Medicine.
Irving H. Leopold	Attending Surgeon and Director, Research Department, Wills Eye Hospital.
Murray F. McCaslin	Profesor of Ophthalmology, University of Pittsburgh School of Medicine.
William E. Krewson	Chief, Department of Motility, Wills Eye Hospital.
William C. Owens	Assistant Professor of Ophthalmology, The Johns Hopkins Hospital.
Raynold N. Berke	Presbyterian Hospital Medical Center.

Otolaryngology:

George J. Thomas	Director, Departments of Anesthesiology, St. Francis' and Medical Arts Center Hospitals, New York.
William C. Huffman	University Hospitals, Iowa City.
Henry L. Williams	Mayo Clinic, Rochester, Minn.
Lee Doerfler	Professor, Audiology Department, Audiology Eye and Ear Hospital, University of Pittsburgh.
Joseph L. Goldman	New York.
Heinrich G. Kobrak	Professor of Otology, Wayne University College of Medicine.

Los Angeles Society of Ophthalmology and Otolaryngology.—The Los Angeles Society of Ophthalmology and Otolaryngology has elected the following officers for 1955:

President, Robert A. Norene, M.D.
Secretary-Treasurer, Leland R. House, M.D.
Chairman of Section on Ophthalmology, Wendell C. Irvine, M.D.
Secretary of Section on Ophthalmology, Stephen J. Popovich, M.D.
Chairman of Section on Otolaryngology, Herschel H. Burston, M.D.
Secretary of Section on Otolaryngology, Ross A. Goodcell, M.D.

CORRECTION

In the article entitled "Chelation of Calcium with Edathamil Calcium-Disodium in Band Keratopathy and Corneal Calcium Affections," by Goodwin M. Breinin, M.D., and A. Gerard DeVoe, M.D. [December, 1954, issue, page 846], there appeared an error in nomenclature. Edathamil calcium-disodium was not the drug used in that study, but rather the authors used the disodium salt of ethylenediamine-tetraacetic acid, adjusted [to neutrality] with sodium bicarbonate.

In the manuscript the term EDTA was used generically for the disodium salt of ethylenediamine-tetraacetic acid. This was regrettable, since it fostered confusion as to the substance used. In accordance with the A. M. A. policy of using [official or] Council-accepted nomenclature only, the manuscript editor unfortunately substituted the term edathamil calcium-disodium. This term actually refers to the end-product of calcium chelation, which would be quite ineffective as a treatment of such conditions.

The exact nature of the chelating substance is complex and deserves future clarification.

We wish to emphasize that the drug used was the disodium salt of ethylenediamine-tetraacetic acid adjusted [to neutrality] with sodium bicarbonate.

GOODWIN M. BREININ, M.D.

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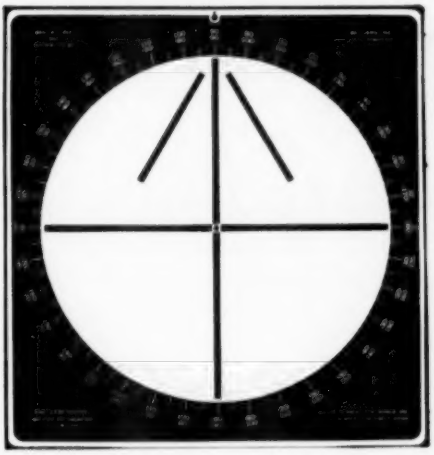

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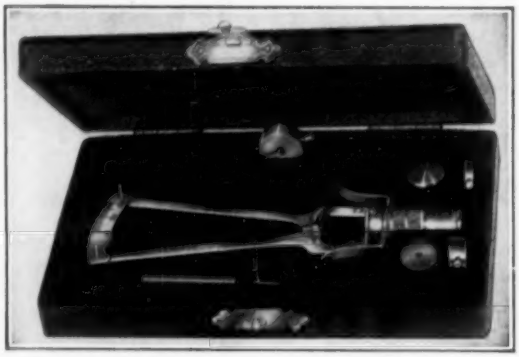

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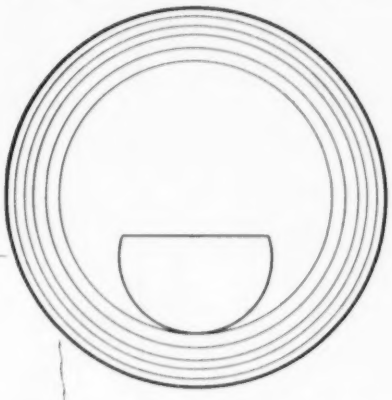

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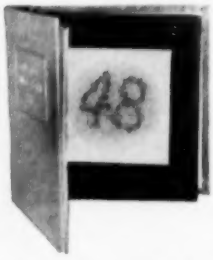

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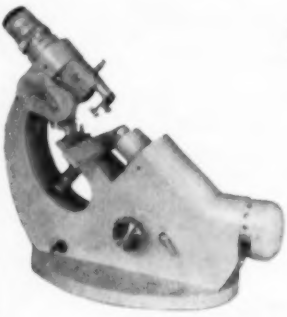

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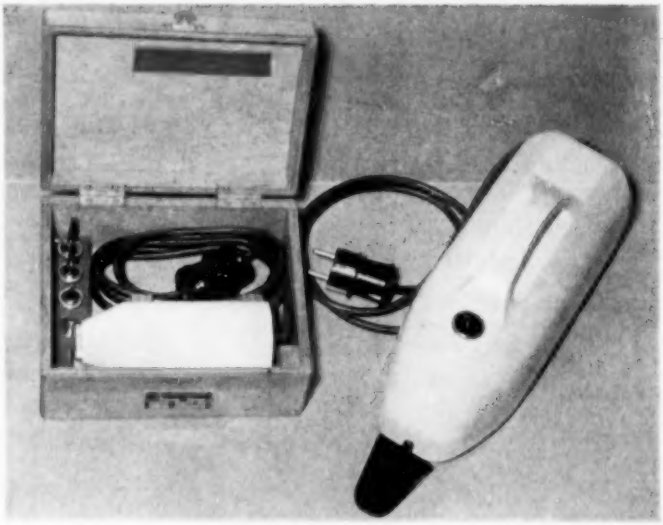

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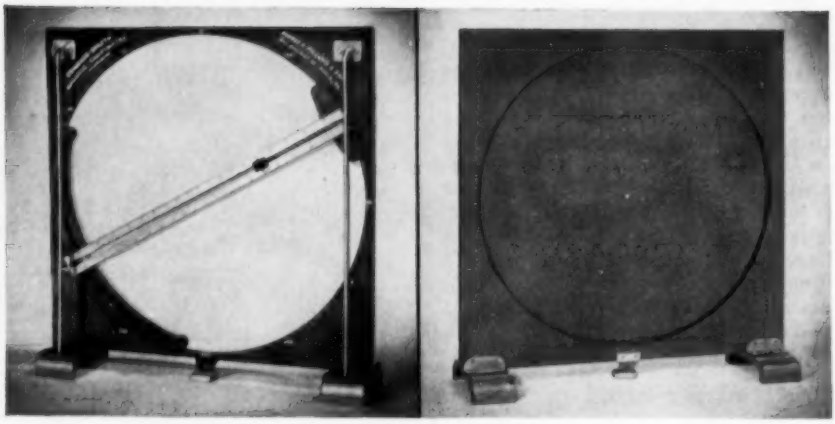

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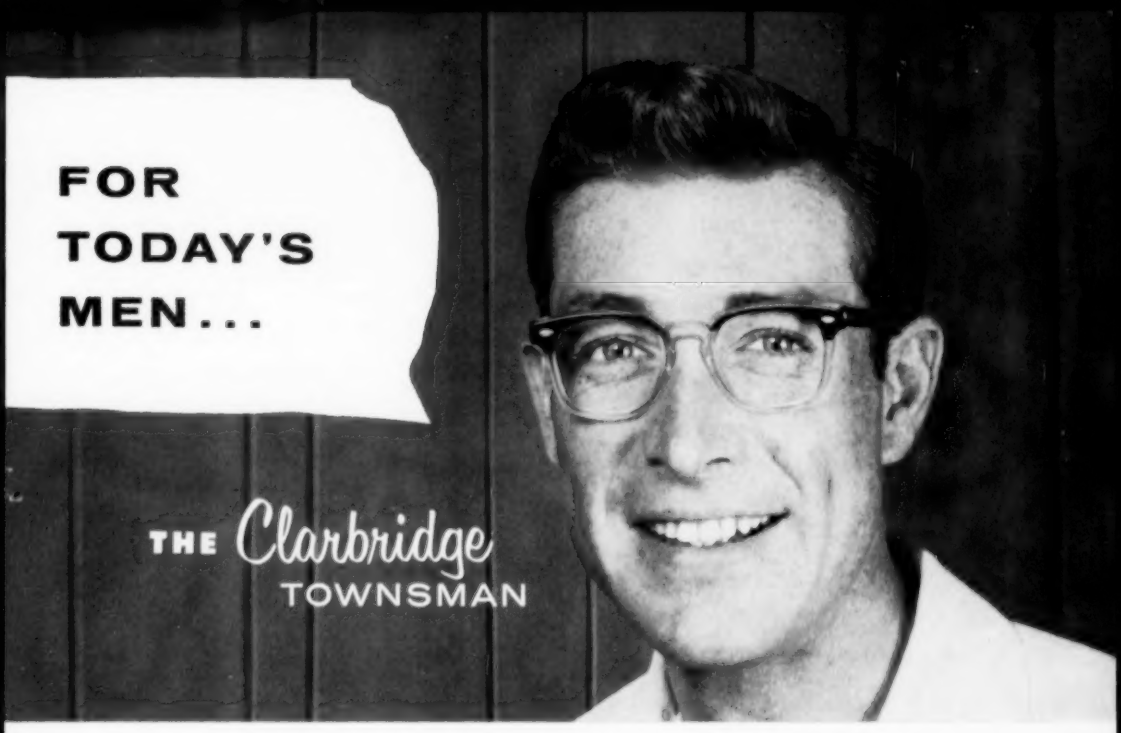

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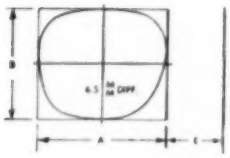

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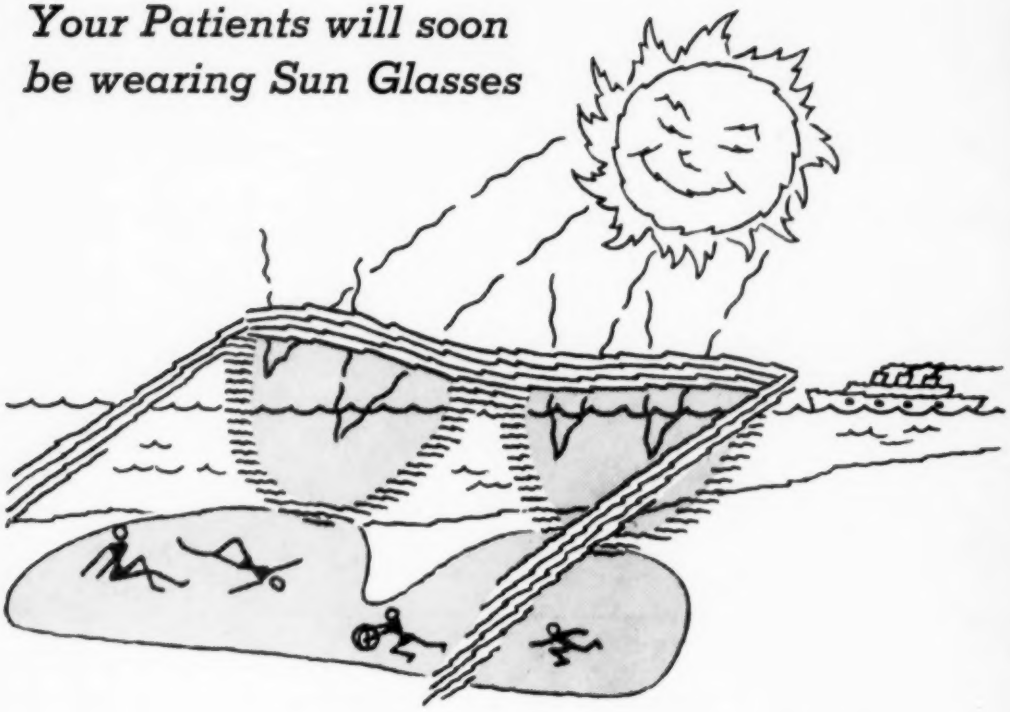

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